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# PREPARATION AND CHARACTERIZATION OF AN ELECTROSPUN GELATIN/ DENDRIMER HYBRID NANOFIBER DRESSING

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# PREPARATION AND CHARACTERIZATION OF AN ELECTROSPUN GELATIN/DENDRIMER HYBRID NANOFIBER DRESSING FOR CHRONIC WOUND TREATMENT

A Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biomedical Engineering at Virginia Commonwealth University

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# Table of Contents

# List of Tables

Page

## List of Figures

Page

# Abstract

PREPARATION AND CHARACTERIZATION OF AN ELECTROSPUN GELATIN/DENDRIMER HYBRID NANOFIBER DRESSING FOR CHRONIC WOUND TREATMENT

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A novel dendritic wound dressing was designed and characterized for its potential to treat chronic wounds. Comprised of gelatin, dendrimer, synthetic polymer and antibiotics, the dressing was electrospun to mimic the natural extracellular matrix (ECM). Gelatin is biocompatible, biodegradable, non-toxic, and easily available. The antibiotic, doxycycline, has the ability to inhibit matrix metalloproteinases. Matrix metalloproteinases, which occur in excess in chronic wounds, degrade the reconstituted ECM. Starburst™ polyamidoamine (PAMAM) dendrimer G3.5, which provides a versatile and structurally controlled architecture to construct nanomedicine, was covalently bonded to the gelatin backbone and electrospun into nanofibers with gelatin, doxycycline and stabilizing polymers. The proposed gelatin/dendrimer hybrid provides a bacterial free

environment and mimics the ECM to promote wound healing. The development of this new polymeric matrix is an important step in advancing the use of bioactive nanofibers with targeted and controlled drug delivery as a wound dressing.

# CHAPTER 1 Introduction

Chronic wounds, also interchangeably termed decubitus ulcers, are one of the major health concerns worldwide. The occurrence of chronic wounds, which are prevalent in elderly patients who are bedfast or patients with poor circulation, paralysis or compromised immune systems, is on the rise.[1] There are a number of causes for this increased incident rate of chronic wounds. Medical advances have resulted in prolonged and improved quality of life for quadric- and paraplegics, as well as individuals with chronic illnesses like diabetes. The increasing elderly population and incidence of diabetes mellitus provide the impetus to achieve cutting edge advances in the treatment and healing of chronic wounds.[2]

The treatment costs of patients with non-healing wounds are significant and necessitate aggressive intervention. This intervention is imperative to decrease morbidity and reduce treatment costs. Yet, the treatment of chronic wounds is both challenging and complex, largely due to prolonged healing resulting from the extended chronic inflammation phase of the wound healing process. This extended phase of chronic wound healing results in infiltration of excessive amounts of neutrophils at the wound site.[3] Neutrophils produce matrix metalloproteinases (MMP), which are destructive proteolytic enzymes responsible for degrading the ECM.[4] Matrix metalloproteinases are also essential to wound healing as they facilitate cell migration and new tissue formation. Too much MMP-8 creates an imbalance and results in degradation of new/regenerated ECM.

Contemporary chronic wound dressings are designed to create and retain a moist environment around the wound bed to facilitate wound healing. [5] Classified by composition, wound dressings include hydrocolloids, alginates, and hydrogels in the form of thin films or fibers, foam sheets and gelatins. Their use is augmented by medicated creams and antibiotic ointments.

The proposed treatment protocol involves the fabrication and application of an electrospun gelatin/dendrimer hybrid having PAMAM G3.5 and encapsulated therapeutic drugs. PAMAM G3.5 is a highly branched structure with surface groups that are easily modified. Doxycycline (DC) is an effective antibiotic with the added ability to inhibit MMPs; thus facilitating wound healing. [6] Gelatin was selected as it is more readily available than collagen. Further, gelatin has many attractive properties as a biomaterial such as its biological origin, biodegradability, and biocompatibility.[7] Four different scaffolds were fabricated for this study. The control scaffold was gelatin [100 mg/mL], and the three evaluation scaffolds were gelatin-dendrimer and encapsulated Doxycycline electrospun with poly(ε-caprolactone), poly(glycolic acid) and poly(lactic-co-glycolic acid), respectively. A major objective of this study was to identify the critical parameters to fabricate a gelatin/dendrimer hybrid with optimum fiber size, mechanical strength and porosity to be used to treat chronic wounds. A secondary objective was to determine if controlled drug release of DC could be achieved using the designed gelatin-dendrimer drug delivery system.

# CHAPTER 2 Background

## 2.1 Chronic Wounds

### 2.1.1 Normal Wound Healing

Wounds are the result of chemical, physical or thermal damage to the skin, blood vessel or other bodily organ. [8] This damage manifests itself as a break or defect in the skin, blood vessel, etc. Illnesses or physiological conditions, such as chicken pox or a hematoma, can also result in a wound. Regardless of whether the damage to the skin is the result of a cut, tear, puncture or contusion from blunt force trauma, the body's response to the injury is convoluted yet systematically organized.

Normal wound healing is usually described using four distinct phases beginning with (1) hemostasis, (2) inflammation, (3) proliferation and (4) remodeling. [9] Hemostasis involves clotting to stem bleeding via a temporary fibrin blood clot. The second phase involves acute and chronic inflammation. Acute inflammation is of short duration and primarily involves phagocytosis of microorganisms and foreign materials by neutrophils. Exudation to provide an influx of fluid, plasma proteins and blood cells to the injured tissue site is also a major activity in acute inflammation. Chronic inflammation is characterized by continued phagocytosis via macrophages that also induce the release of neutral proteases, chemotactic factors, reactive oxygen metabolites and growth factors. Connective tissue and blood vessel proliferation is also initiated. In the proliferation phase, transforming growth factor beta (TGF-β) yields increased transcription of genes for synthesis of collagen, proteoglycans and fibronectin; but most importantly decreases the

protease responsible for the breakdown of matrix proteins. [10] The final phase, remodeling, is characterized by the synthesis and degradation of ECM components to establish a new equilibrium.

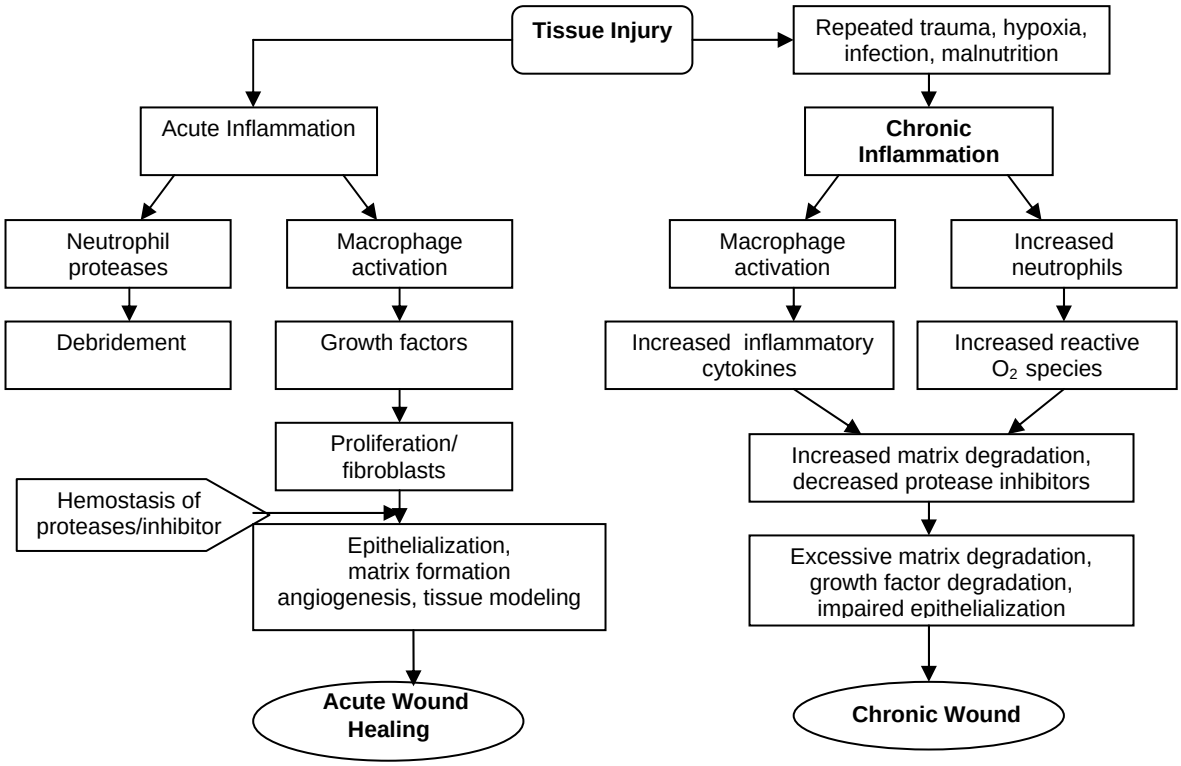
### 2.1.2 Chronic Wounds and Matrix Metalloproteinases

Chronic wounds represent a significant cause of morbidity and mortality for adult patients experiencing diabetes, cancer, malnutrition, physical disabilities (e.g. paralysis), and compromised immunity due to illnesses like HIV/AIDS. [11] Non-healing wounds do not progress through the stages of normal wound healing due to the self-sustaining cycle of chronic inflammation leading to the destruction of the ECM. [12] Matrix metalloproteinases are enzymes that are involved in tissue reorganization, inflammation and remodeling. Sixteen MMPs have been identified and characterized for substrate specificity. [13] Failure of MMP regulation has been correlated with deficient wound healing, such as chronic wound healing. Chronic non-healing ulcers were characterized by significantly higher levels of MMP-1 and MMP-8 and lower levels of tissue inhibitor of metalloproteinase 1 (TIMP-1) in a comparison of chronic non-healing ulcers to normal healing wounds. Studies have shown that the over-expression and activation of neutrophil-derived MMP-8 is involved in the pathogenesis of nonhealing chronic ulcers. [14]

Wounds are classified as either acute or chronic. Acute wounds usually result from disruption to the tissue, yet heal completely in the expected time-frame of eight to 12 weeks, and with minimal scarring[12]. However, chronic wounds that develop from tissue injuries are slow to heal, and have not healed within the expected 12 week timeframe.

Chronic wound healing is impeded by repeated tissue insults or underlying physiological conditions like diabetes, poor blood circulation or persistent infections[15]. Chronic wounds are impeded by extended inflammation where the balance of ECM degradation and synthesis is compromised by the excessive amounts of neutrophils thus generating increased levels of MMPs. Figure 1 is a schematic adapted from Nwomeh et al., summarizing key differences in normal and chronic wound healing. [12] As noted in the diagram, the amount of neutrophils increases, therefore increasing matrix degradation due to MMP. Additionally, protease inhibitors are decreased, which allows even further matrix degradation. The degradation occurs not only of the damaged ECM and tissue, but reconstituted matrix is also degraded.

**Figure 1**: Normal versus Chronic Wound Healing



### 2.1.3 Healing and Treatment

The majority of chronic wounds are pressure ulcers (bed stores), diabetic foot ulcers and venous stasis ulcers, with the latter accounting for 70% of chronic wounds. [16] A pressure ulcer is a break in the skin and is commonly located in the posterior and ischial sections of the pelvis, the heel or the upper part of the femur. Pressure ulcers are caused by ischemia that occurs when pressure on the tissue is greater than the pressure in capillaries, thus restricting blood flow into the area. [17] They occur due to friction and external pressure in the elderly, and patients that are bed-ridden or have spinal cord injuries/paralysis. Chronic wounds develop in four distinct stages. [18] These four stages influence the treatment protocol and are listed in Table 1.

**Table 1**: Four Distinct Stages of Chronic Wound Progression

| Stage | General Characteristics |
|---|---|
| Stage I | Reddened area on the skin; no break. Non-blanchable when pressed; and coloration is pink, red, or mottled after pressure is relieved. Patients with darker pigmentation have subtle purplish or extra discoloration. |
| Stage II | Partial loss of skin thickness. Skin blisters and forms an open sore. The area is broken, cracked, blistered, and mottled in color. Necrotic tissue or drainage may also be present. |
| Stage III | Full loss of skin thickness. Skin breakdown (i.e. crater); tissue damage may be necrotic (black), draining (yellow), or granulating (red). |
| Stage IV | Deep pressure ulcer, muscle, bone tendon or joint damage. Damage may have extensive drainage and necrotic tissue. |

Current and emerging chronic wound treatments are designed to alleviate some of the causes responsible for delayed healing. Methods for wound repair include mitigation of the primary causes like external pressure, ischemia, bacterial load and protease imbalance. The application and use of antibiotics and antibacterial agents, debridement, irrigation, vacuum-assisted closure, oxygenation, moist wound healing and addition of cells and growth factors are various methods used to advance healing of chronic wounds. [19]

The treatment protocol for Stage I pressure ulcers involves decreasing or eliminating the external pressure source, reducing moisture from incontinence, employing pressure relief devices, and ensuring adequate patient hydration and nutrition. To prevent bacterial invasion, transparent film, foam or hydrocolloid dressings are applied.[20] The primary function of the temporary dressings is to serve as a barrier, much like human skin. Dressings to treat Stage II ulcers are principally designed to keep the peri-ulcer skin dry while maintaining a moist wound bed. Topical antibiotics and antibacterials like silver sulfadizine, doxycycline or triple antibiotic are applied to cure or prevent infection. [21-22] Debridement, irrigation, and warming are also employed to decrease bacterial load. The healing of chronic wounds is further compromised by the failure of fibroblasts to produce adequate ECM proteins. [23] Various methods exist to balance proteases through implantation of cells, growth factors, hormones and other materials to secrete or enhance healing. Wound pain management through use of analgesics and vitamin and mineral supplementation is also important in the resolution of chronic wounds. Bromelain, vitamins A, C and E, zinc, and glucosamine are some of the supplemental vitamins and minerals used to promote wound healing.

## 2.2 Dendrimers for Drug Delivery

Dendrimers are an inimitable class of polymeric nanoparticles that play key roles in emerging nanotechnology, biotechnology, and nanomedicine. Dendrimers are spheroid or globular nanostructures that are precisely engineered to carry molecules encapsulated in their interior void spaces or attached to the surface. [24] Dendrimers are attractive in the field of nanomedicine as drug carriers for reasons such as:

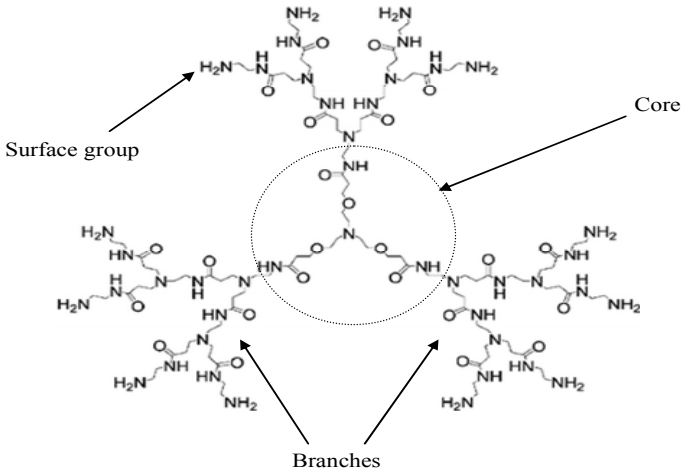
1. Low polydisperity index
2. Multiple attachment sites
3. A well-defined, controllable size
4. A highly-branched globular structure that can be easily modified to change its chemical properties.
5. Capability of drug encapsulation in the core of the dendrimer as well as dendrimer-drug conjugation by drug attachment to the surface of the dendrimer.

The use of dendrimers for drug delivery and controlled drug release improves drug efficacy. Controlling the release time and delivery location of therapeutic drugs allows sustained and targeted drug delivery in the therapeutically desirable range; thus contributing to lower drug dosages and a reduction of harmful side effects.

### 2.2.1 Structure of PAMAM Dendrimers

Dendrimers are large, complex macromolecules with well defined and consistent size and form. Their highly branched, three-dimensional architecture consists of a core, branches and end groups. Figure 2 is a schematic of a PAMAM (G2) dendrimer.

**Figure 2**: Chemical Structure of PAMAM (G2.0) Dendrimer




Size, shape, and reactivity of dendrimers are determined by generation (branch levels) and chemical composition of the core, interior branching, and surface functionalities. The terminal groups heavily influence the chemical properties of the molecule and physical properties such as solubility and viscosity. [25] Dendrimers with hydrophilic surface groups are soluble in polar solvents and vice-versa. In solution, linear polymer chains exhibit flexible coils and dendrimers in comparison reflect a tightly coiled ball affecting their viscosity.

The multi-functionalized core of the dendrimer forms the central point of the molecule. The monomer chains that branch out from the core are termed the first generation, (G1.0); where the generation increases for every two monomer branches. Dendrimer surface area and size of the molecule increases with each generation- until the effect of steric hindrance prevents continued growth (branching). Dendrimers become

densely packed as they extend out to the periphery forming a closed membrane-like structure. At this juncture, dendrimers are said to have reached a critical branched state called the "starburst effect". [25] The starburst effect is witnessed after the tenth generation for PAMAM dendrimers.

The shape of the dendrimer is determined by its generation. Dendrimers of lower generations (G0-2) are asymmetrically shaped and contain more inner void space (internal cavities) as compared to higher generations. Higher generation dendrimers (G4 and greater) are globular shaped. [26]

2.2.2 Synthesis of PAMAM Dendrimers

Dendrimers are constructed through a set of repeating chemical synthesis procedures that build up from the molecular level to the nanoscale region. Dendrimers are generally prepared using divergent or convergent methods. Both methods involve iterative sequences in which each iteration leads to a higher generation dendrimer. In the divergent method, the dendrimer grows outward from a multifunctional core. The core reacts with monomers containing one reactive and two dormant groups giving rise to the first generation. [26] The dendrimer diameter increases linearly whereas the number of surface groups increases geometrically. Disadvantages of the divergent method are side reactions and incomplete reactions of the end groups causing defects in the dendrimer structure. Additionally, the divergent method requires a large excess of reagents to drive reactions to completion and prevent side reactions. This makes purification more difficult.

The convergent method resolves difficulties in the divergent method of dendrimer synthesis. The convergent method sequentially builds the dendrimer from the end group inwards. The method is initiated with the attachment of growing branched polymeric arms (dendrons) to a multifunctional core molecule. Advantages of this approach include easy end-product purification and minimal defects. The convergent method is limited in that steric problems occur in the reactions of the dendrons and core molecule. [26]

PAMAM dendrimers were the first dendrimers synthesized by Donald Tomalia and co-workers in the 1980's. Ammonia is used as the core molecule. [27] In the presence of methanol, ammonia reacts with methyl acrylate then ethylenediamine is added. This allows ethylenediamine to also be used as a core molecule. The free amino group at the end of each branch reacts with two methyl acrylate monomers and two ethylenediamine molecules to form successive generations. Half-generation PAMAM dendrimers have tertiary anionic carboxylate groups; and for full generation have cationic amino terminal groups.

### 2.2.3 Drug Delivery Mechanisms

Drug delivery systems are employed to increase the efficacy of therapeutic drugs through locally targeted and sustained release of a drug over time. Two common drug delivery systems are liposomes and polymeric systems, yet both are limited. Linear polymers are polydisperse; therefore the molecular weight distribution impacts *in vivo* biodegradation. Liposome-based systems have poor stability and difficulty targeting specific tissues.

Three main paradigms employed for dendrimer drug delivery are drug encapsulation, dendrimer-drug conjugation and lyposomes. Encapsulation of drugs uses the steric bulk and internal cavities for entrapping hydrophobic drugs at the core. Dendrimer-drug conjugates are generated via covalent attachment to the dendrimer surface groups. For example, dendritic unimolecular micelles can be fabricated through PEGylation on the dendrimer surface. [26-28] PEGylation stabilizes the entrapped drugs, provides additional sites to retain more drugs and increases the solubility of the entrapped drugs.

Regardless of the drug carrier design, the ideal drug delivery system should be inert, biocompatible, achieve high drug loading and increase solubility at physiological conditions. The use of PAMAM dendrimers for the design of drug delivery systems is largely due to desired *in vivo* biological properties such as non-toxicity and non-immunogenicity . [26]

2.3 Poly (α - hydroxy esters)

Poly(α-hydroxy esters) are the most commonly used synthetic polymers in tissue engineering applications. [29] As a biomaterial, poly (α-hydroxy esters) such as poly(ε-caprolactone) (PCL) , poly(glycolic acid) (PGA) and the copolymer poly(lactic-co-glycolic acid) (PLGA), are desirable for their biocompatibility and biodegradation properties. Electrospinning the aforementioned synthetic polymers with natural polymers like collagen, gelatin, elastin, etc., increases the mechanical integrity and strength of the fibrous scaffold. The biocompatibility of PGA, PLGA, and PCL copolymers, affirms their

preference as tissue engineered (TE) scaffold applications as they minimize the chronic foreign body reaction and facilitate tissue regeneration.[30]

The general selection criteria for the polymers used for this study was based on the mechanical properties and the time of degradation. Key polymer properties are listed in Table 2. Some of the benefits of the polymers used in this study are as follows:

- Mechanical properties that remain strong until surrounding tissue has healed.
- Do not invoke an inflammatory or toxic response.
- Metabolized in the body after fulfilling their purpose, leaving no trace.
- Easy processing into final product form.

**Table 2**: Polymer Properties

| Polymer | Melting Point (°C) | Glass-Transition Temp (°C) | Modulus (Gpa)[a] | Crystallinity (%) | Degradation Time (months)[b] |
|---|---|---|---|---|---|
| PCL | 58—63 | (-65)— (-60) | 0.4 | 30-70 | >24 |
| PGA | 225—230 | 35—40 | 7.0 | 50 | 3 to 6 |
| PLGA 85:15 (ratio dependent) | 173—230 | 40—60 | 0.2 | Amorphous | 6 to 12 |

a) Tensile or flexural modulus. b) Time to complete mass loss.

2.3.1 Poly(ε-caprolactone) (PCL)

Poly(ε-caprolactone) is a biodegradable polyester prepared by ring opening polymerization of ε-caprolactone. Because PCL is degraded by hydrolysis of its ester linkages in physiological conditions, it has received extensive interest for construction of

implantable biomaterials and drug delivery devices. The ring-opening polymerization of ε-caprolactone yields a semi-crystalline polymer with a melting point of 59—64°C and a glass-transition temperature of -60°C. Biodegradation of PCL *in vivo* can take up to 30 months. [31] Because the homopolymer has a degradation time greater than 2 years, copolymers have been synthesized to accelerate the rate of bioabsorption. PCL has been commercially developed as a resorbable suture, adhesion barrier, and used for TE scaffolds. [32] PCL beads are also used to encapsulate drugs for targeted and controlled drug release.

2.3.2 Poly (glycolic acid) (PGA)

Poly(glycolic acid) is a biodegradable, thermoplastic polymer and the simplest linear, aliphatic polyester. Poly(glycolic acid) is usually prepared by polycondensation or ring-opening polymerization. [33] Mostly soluble in highly fluorinated solvents like hexafluoroisopropanol (HFIP), high MW PGA polymer has been used in melt spinning, electrospinning and film preparation. Poly(glycolic acid) undergoes hydrolytic degradation in physiological environments; which is augmented by enzymatic breakdown through esterase activity. Poly(glycolic acid) degrades into glycolic acid, which is a naturally occurring byproduct in the metabolic pathway. As such, glycolic acid is non toxic and excreted in urine and as water and carbon dioxide. [32] In vivo degradation of biomaterials synthesized with PGA is usually short; and degrades within seven days. Previous experimental studies of sutures made with PGA showed a 50% loss in material strength after two weeks; and complete loss of strength after four weeks. [34]

One of the first biomedical applications for PGA was as a resorbable suture marketed in 1962 as Dexon™. [35] Because PGA yields strong fibers that degrade into water soluble monomers- it has found widespread use in surgical applications. Implantable medical devices (e.g. rings, pins, rods, plates, screws, etc.) are also produced with PGA. The use of PGA as a biomaterial has evolved to fabrication of TE scaffolds and for controlled drug delivery.

### 2.3.3 Poly (lactic-co-glycolic acid) (PLGA)

Poly(lactic-co-glycolic acid) is a copolymer synthesized by ring-opening co-polymerization of two different monomers, the cyclic dimers (1,4-dioxane-2,5-diones) of glycolic acid and lactic acid. [36] Successive monomer units of glycolic acid and lactic acid are linked together via ester bonds to form PLGA during polymerization. Different compositions of PLGA can be formed by altering the ratio of lactide to glycolide. For example, PLGA 85:15 is comprised of 85% lactic acid and 15% glycolic acid. PLGA can be dissolved by general solvents, such as tetrahydrofuran, acetone, chlorinated solvents and ethyl acetate. [37]

In the presence of water and in physiologic conditions, PLGA degrades by hydrolysis of its ester linkages. The degradation rate is dependent on the monomer ratios. The more glycolic acid units present, the higher the polymer solubility thus resulting in faster degradation. An exception to this rule is the copolymer with a 50:50 monomers' ratio, which exhibits the faster degradation (about two months). [37]

The in vivo degradation of PLGA yields the original monomers, lactic acid and glycolic acid. Lactic acid and glycolic acid are by-products of various metabolic pathways in the body. [36] As a result, the use of PLGA for drug delivery and biomaterial applications causes minimal toxicity. The ability to regulate polymer degradation rate by controlling the polymer monomer composition makes the use of PLGA in biomaterial applications highly appealing. PLGA is used to construct various biomaterials like grafts, sutures, implants, prosthetic devices, and micro and nanoparticles.

## 2.4 Wound Dressings

Wound dressings play an essential role in the treatment and healing of chronic wounds. Key functions of dressings for healing chronic wounds include:

1. Protecting the ulcer from further injury,
2. Preventing infection and invasion of bacteria and other germs,
3. Maintaining the proper environment for healing,
4. Filling in the wound's void space.

Wound dressings, unlike gauze or bandages, aid normal healing by maintaining moisture in the wound bed. A chronic wound has a better chance of healing when the moisture level is similar to that of healthy skin. Wound dressings are classified based on their function in the wound, material type, physical form and contact layer level. [38] Categories are highlighted in Table 3.

**Table 3**: Various Categories to Classify Dressing Type

| Category | Different Classes |
|---|---|
| Wound Function | debridement, antibacterial, occlusive, absorbent, adherence |
| Material Type | hydrocolloid, alginate, collagen, hydrogel, etc. |
| Physical Form | ointment, film, foam, gelatin |
| Contact Layer | primary dressing (direct wound contact), secondary dressing (cover primary dressing), island dressing (have a central absorbent region surrounded by an adhesive portion) |

2.4.1 Traditional and Emerging Dressings

Wound dressings have evolved over the years from mere coverings (plant herbs, cotton wool, bandages and gauze) to bioactive, tissue-engineered (TE) scaffolds that dispense therapeutic agents in a controlled manner and facilitate tissue regeneration via interaction with cells. Disadvantages of conventional dressings (e.g. cotton wool, gauze) for treating chronic (open) wounds include delayed wound healing and generation of more damage.[39] Gauzes are mainly used in chronic wounds as packing stuff to absorb fluid and exudates; thus drawing fluid away from the wound. Some of the major disadvantages of using traditional dressings for chronic wound management include:

1. Loss of bacterial protection when the outer surface of the gauze dressing becomes moistened by wound exudates or external fluids.
2. Adherence of the dressing to the wound as fluids decrease. The dressing becomes painful to remove and dries out the wound.
3. Requirement of frequent change out which diminishes patient compliance.

Contemporary dressings have been developed to address the shortfalls of traditional dressings. One of the most widely used dressings for chronic wounds are hydrocolloids. [38] They are designed to be used for light to moderately exuding wounds such as pressure sores, leg ulcers, minor burns and traumatic injuries. Hydrocolloid dressings are impermeable to water vapor in their intact state. However, upon absorption of wound exudates, the physical state of the dressing transforms to a gelatin. As the gelatin forms, it becomes more permeable to water and air. [40] The gelatin is not painful to remove, thus contributing to its popularity as a pediatric dressing. Table 4 summarizes the top dressings used to treat chronic wounds, as well as some of the advantages and disadvantages of each. [41]

A new generation of bioactive and medicated wound dressings that mediate and regulate cell interaction are currently being developed, which is the focus of this study. This milestone evolution advances the following in wound care management:

1. The fabrication of wound dressings from natural or biodegradable synthetic polymers (collagen, gelatin, chitosan, PGA, PLGA, PCL) that mimic the tissue matrix thus promoting cell attachment and proliferation.
2. The application of medicated TE scaffolds that yield targeted and controlled release of antibiotics, antimicrobials, vitamin and mineral supplements and growth factors that accelerate wound healing.

**Table 4**: Different Dressings for Chronic Wound Management

| Dressing | Advantages | Disadvantages |
|---|---|---|
| Hydrocolloids | Impermeable to bacteria | Impermeable to oxygen |
| (light to moderate exudates) | Can be left in place for up to 7 days without changing | Cannot handle heavily exudating wounds |
| | Gelatin creates moist wound bed | Gel & smell phenomenon |
| Hydrogels | Soothing to patient | Need frequent changing |
| (dry and painful wounds) | Maintains intimate contact with the wound surface | Heavily exudating wounds causes leakage |
| | Creates moist environment for cell migration | Cost |
| Alginates | Highly absorbent | Fibrous debris, lateral wicking |
| (moderate to heavy exudates) | Readily biodegradable; form gelatin upon exudates absorption | Requires moisture to function; thus cannot be used for dry wounds |
| | Impermeable to bacteria | |
| Foams | Absorbent | Smelly, malodorous discharge |
| (light to moderate exudates) | Occlusive | Opaque |
| | Thermal insulation | |
| | Change every 3 days | |

2.4.2 Electrospun Gelatin/Dendrimer Hybrid with Doxycycline

Natural and synthetic polymeric wound dressings not only cover and protect the wound from bacteria and further damage, they can also serve as drug delivery systems and bioactive platforms to facilitate tissue growth. Upon hydration by the wound exudates,

drug delivery is achieved via hydrolysis, diffusion or gelatin formation and release followed by degradation of the dressing/erosion of the gelatin. The localized and controlled release of antibiotics is preferred over systemic delivery as it allows direct targeting of the wounded area and metered drug administration over an extended period of time.

Doxycyline has been selected as the antibiotic to include in this study. It is an effective antibiotic that has the ability to inhibit the degradative enzyme, MMP. [6] Doxycycline is a member of the tetracycline antibiotics group, commonly used to treat infections. Tetracyclines work by inhibiting protein synthesis in bacteria by binding to the 30S ribosome, immobilizing the progressive translation of proteins thus preventing reproduction. It is hoped that this mechanism of action will mitigate the formation of biofilms that routinely develop in chronic wounds. Doxycycline is frequently used to treat inflammation of the prostate, respiratory and urinary tract infections, syphilis, pelvic inflammatory disease, acne and Lyme disease.[42] It is also used to prevent and treat infections from E. Coli and methicillan-resistant Staphyloccus aureas (MRSA).

Tissue-engineered scaffolds are used to direct the growth of cells that are either seeded within the porous structure of the scaffold or migrating from surrounding tissue. [43] The regeneration of tissue, i.e. wound healing, can also be supported by growth factors and supplements that are contained in the dressing. Electrospinning is recognized as an efficient processing method to manufacture TE scaffolds that mimic native ECM. [44] The originality of the proposed wound dressing is based on combining these technologies to

design an electrospun gelatin/dendrimer hybrid for the treatment of chronic ulcers. Uniqueness of the proposed dressing includes:

1. Fabrication of an electrospun drug delivery platform made from the natural biopolymer gelatin (denatured collagen).
2. Nano-electrospinning gelatin with copolymers PGA, PLGA, and PCL to enhance mechanical strength and ensure biodegradability.
3. Controlled and targeted drug release of Doxycycline that facilitates wound healing as an antibiotic and by inhibiting the degradative enzyme,MMP.

The advantages and disadvantages of the proposed dressing compared to dressings currently on the market are summarized in Table 5.

**Table 5**: Proposed versus Contemporary Wound Dressings [41]

| DRESSING | ADVANTAGES | DISADVANTAGES |
|---|---|---|
| Foams | ▪ Highly absorbent<br>▪ Debrides wound<br>▪ Comfortable & conformable | ▪ Requires secondary dressing<br>▪ Cost<br>▪ Cannot be used on dry wounds |
| Hydrogels | ▪ Soothing to patient<br>▪ Intimate contact with wound surface<br>▪ Creates moist environment for cell migration | ▪ Need frequent changing<br>▪ Cost<br>▪ Cannot handle heavily exudating wounds without leakage |
| Hydrocolloids | ▪ Impermeable to bacteria<br>▪ Can be left in place for extended periods of time<br>▪ Gel creates moist wound | ▪ Impermeable to oxygen<br>▪ Cannot handle heavily exudating wounds<br>▪ Gel & smell phenomenon |
| Proposed Dressing | ▪ Controlled release of therapeutic drugs<br>▪ Inhibition of destructive enzymes (MMP)<br>▪ Maintains moist wound bed | ▪ Pure electrospun gelatin is mechanically weak<br>▪ Required to cross-link or electrospin with stabilizing polymers (PCL, PGA, PLGA) |

# CHAPTER 3 Experimental Materials and Methods

## 3.1 Materials

**Table 6:** List of Materials

| Material | Abbreviation |
|---|---|
| 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide | EDC |
| 4-dimethylaminopyridine | DMAP |
| 4-nitro phenyl chloroformate | NPC |
| Chloroform | |
| De-Ionized Water | DI water |
| Deuterium oxide | $D_2O$ |
| Dichloromethane | DCM |
| Dimethylformamide | DMF |
| Doxycycline | DC |
| Ethanol (denatured) | |
| Ethyl ether (anhydrous) | |
| Hexafluoroisopropanol | HFIP |
| Hydrochloric Acid | HCl |
| N,N-Dicyclohexylcarbodiimide | DCC |
| N-Hydroxy Succinimide | NHS |
| Ninhydrin | |
| Poly (Glycolic Acid) | PGA |
| Poly (Lactic-Co-Glycolic Acid) (85:15) | PLGA |
| Poly(ε-Caprolactone) | PCL |
| Porcine type-A gelatin | Gelatin |
| Sodium carbonate | $NaHCO_3$ |
| Starburst™ G3.5 PAMAM dendrimer | G3.5 |
| Tetrahydrofluran | THF |
| Triethylamine | TEA |

3.2 Equipment

**Table 7:** List of Equipment

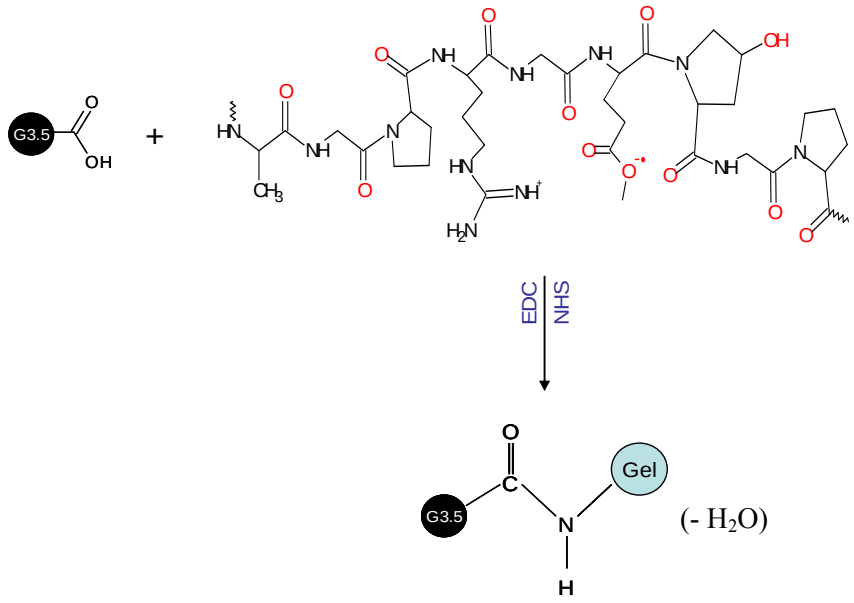
| Name | Purpose |
|---|---|
| MTS Bionix 200® Mechanical Testing System | Measure the tensile properties (stress, elongation) of electrospun fibers. |
| Scanning Electron Microscope Model 550 | Photographs of scaffolds (characterize morphology and fiber diameter using ImageTool™). |
| Eppendorf Centrifuge Model: 5415D | For Centrifugation, separation of the suspended phase form liquid phase. |
| Freeze Dry System – Labconco Model 77550 | Freeze dry system to dry the frozen samples. |
| Nuclear Magnetic Resonance (NMR) | Proton NMR measurement was performed on a 300 MHz NMR spectrometer. |
| Permeability Test Mechanism | Designed and fabricated by Scott Sell to measure permeability (see section 3.4.6). |
| Ultra Violet – Visible (UV-Vis) Spectrophotometer Genesys 6 | Quantitative analytical tool for Ninhydrin assay and release experiments. |
| UV Radiation source | UV light source, 90 W high-pressure mercury vapor filled lamp, manufactured by Phillips (Holland). |
| Weighing Balance | For measuring the required amount of materials. |

### 3.3 Synthesis

#### 3.3.1 Conjugation of Gelatin to PAMAM Dendrimer G3.5

The reaction synthesis for conjugating PAMAM G3.5 to gelatin is represented in Figure 3. The reaction was carried out in three phases: formulate the gelatin solution, activate the terminal carboxyl groups of the dendrimer and lastly, conjugate dendrimer to gelatin. The gelatin solution was prepared by weighing and adding 20 mg of gelatin (type A Porcine powder) to 20 mL of sodium carbonate (pH 8.5). The mixture was stirred at 80°C until the solution was clear.

**Figure 3**: Conjugation Reaction of Gelatin to G3.5 Dendrimer




Conjugation of gelatin to G3.5 involved the activation of the carboxyl (-COOH) groups of the dendrimer using EDC chemistry to form a stable amide bond. Prior to the reaction, 0.75 µmol of PAMAM G3.5 was dried via rotary evaporation to remove the storage solution (i.e. methanol). The obtained dry product was then dissolved in 2 ml of DI

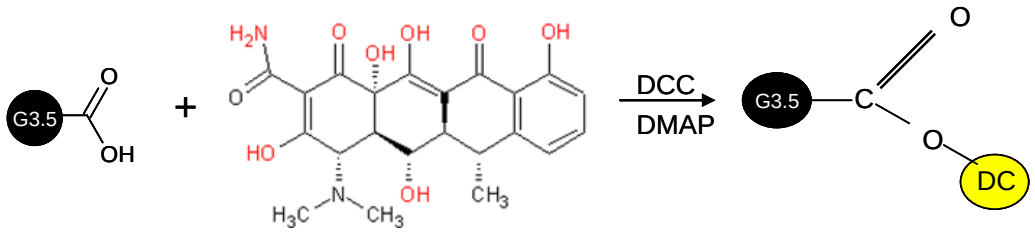
water; added in 1mL increments and vortexed to ensure complete removal of PAMAM G3.5 from the evaporator flask and transferred to a scintillation vial. PAMAM G3.5 has 64 carboxyl surface groups (S.G.).[45] The EDC reagent was kept at a minimum to only activate half (32) of the surface groups. The remaining surface groups were reserved for subsequent drug attachment. To this solution, 3mg of NHS and 5mg of EDC were added and then stirred for 24hr at room temperature for activation of the surface groups. The activated G3.5 solution was mixed with the gelatin solution and stirred in an ice bath for 4 hr. The salt was removed by centrifugation. The supernatant obtained was then added drop-wise to 50 mL of cold ether and kept at -8 ºC for 24 hrs. The precipitate was collected and then dried via rotary evaporation. Rapid dialysis (12-14000 MWCO dialysis tubing; DI water as the filtrate) was carried out to remove un-reacted NHS and EDC for purification of the product. The recovered product was lyophilized for 24 hr to dry the sample and then weighed.

### **3.3.2** Conjugation of Doxycycline to PAMAM Dendrimer G3.5

Conjugation of DC to PAMAM G3.5 involved the activation of carboxyl (-COOH) groups of the dendrimer using HCl. Prior to the reaction, 0.75μmol of PAMAM G3.5 was dried via rotary evaporation. The obtained dry product was then dissolved in 1 ml of DI water. The solution was acidified with three to four drops of 1 N HCl (pH=1.0). The acidified solution was dried again by rotary evaporation and then re-dissolved in 2 ml of DMF (added at 1mL increments, vortexed and transferred to a scintillation vial). To this solution, 15mg of DC, 8.5mg of DCC and 3.4mg of DMAP were added. The solution was

stirred for 14 hrs at 4 ºC in an ice bath. After stirring, the solution was added drop wise to 40 mL of cold ether and kept at -40 ºC for 24 hrs to precipitate the salt (DCU). The mixture was centrifuged to remove the precipitate. The supernatant was dried by means of rotary evaporation. After drying, dialysis- with DI $H_2O$ as the filtrate, was performed to remove non-reacted DCC and DMAP for further purification of the product. The recovered product, G3.5 conjugated to DC, was analyzed by $^1$H-NMR. We could not confirm the synthesis of DC to PAMAM G3.5. As this reaction did not yield the desired result, DC was encapsulated in the spinning solutions and electrospun into a gelatin/dendrimer hybrid.

**Figure 4**: Proposed Conjugation Reaction of DC to G3.5 dendrimer




### 3.3.3 Encapsulation of Doxycycline

Twenty milligrams of DC powder and 10 mg of PAMAM G3.5 were measured and dissolved in 5mL of HFP along with various weight ratios of gelatin and the polymers included in this study. It was assumed that as the melt polymer solutions were ensured to be homogenous prior to electrospinning, that DC and PAMAM G3.5 would also be evenly distributed with the other contents (gelatin and synthetic polymers) in the electrospun mat.

### 3.3.4 Polymer-Gelatin Spin Dope Solutions

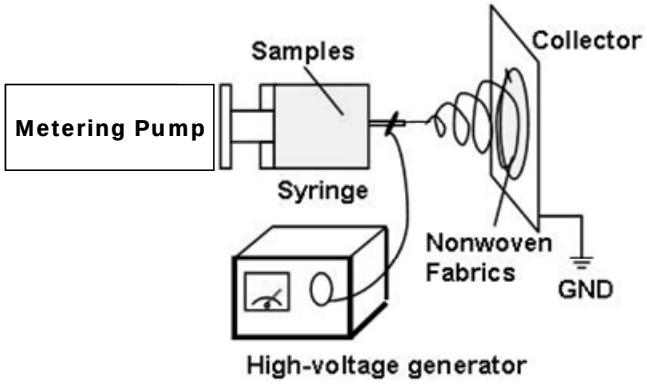
Solutions of 100 wt % gelatin, 50/50 (w/w) and 70/30 (w/w) PCL/gelatin, 50/50 (w/w) and 70/30 (w/w) PGA/gelatin and 50/50 (w/w) and 70/30 (w/w) PLGA/gelatin were prepared by dissolving 500mg total in 5mL of HFIP in a glass scintillation vial. All reagents were purchased from Sigma Aldrich Corporation and the synthetic polymers were purchased from Lakeshore Biomaterials. Solutions were mixed for 24h on a shaker plate to ensure complete dissolution to form a homogenous solution. The solutions were loaded into a 5 mL Beckton Dickinson syringe and placed in a KD Scientific syringe pump (Model 100) for electrospinning.

### 3.3.5 Electrospinning

Electrospinning is a type of extrusion type fiber fabrication method that employs the use of an electrical charge to product micro and nano-scaled fibers from a liquid. The experimental setup used for the electrospinning process is schematically shown in Figure 5. To form fibers during electrospinning, a polymer solution is loaded into a syringe and forced to the needle tip by a syringe pump. A positively charged electrode is attached to the needle and when voltage is applied, a static electric field is created causing the droplet of polymer at the end of the syringe to be stretched into a conical shape known as the Taylor cone. With increasing electrical field, the discharged polymer solution jet is elongated and whipped continuously by electrostatic repulsion causing the solvent to evaporate. The charged polymer fiber is deposited on a grounded collector. For this study, an adjustable high voltage power supply (Spellman CZE1000R; Spellman High

Voltage Electronics Corporation) was used to apply a 25kV voltage to a blunt tip 18 gauge needle fixed to the solution containing syringe. The negative electrode was connected to a metallic lab rack with its circular plateau wrapped in aluminum foil. The spin solution was delivered via a syringe pump controlling the mass flow rate to 3.5 mL/hr. Solutions were electrospun onto a flat, stainless steel, grounded mandrel (2.5 x 10.2 x 0.3 cm) placed approximately 12 cm from the needle tip and rotated at 500 rpms with back and forth traversing to produce a scaffold of randomly oriented fibers; evenly distributed along the collector mandrel. Upon completion of the spinning process, the scaffolds were removed from the mandrel (sides slit; then gently peeled); and placed in the fume hood for degassing and removal of HFIP. Samples were then stored for 24h in the dessiccator to ensure moisture removal.

**Figure 5**: General Electrospinning Apparatus



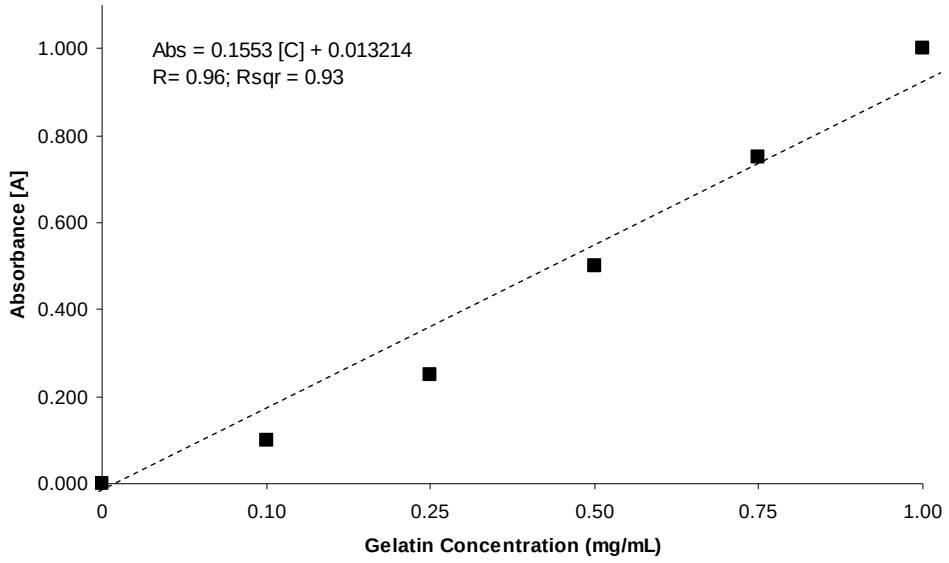

### 3.4 Characterization

3.4.1 Ninhydrin Assay

Verification of the conjugation of gelatin to G3.5 was indirectly determined by measuring the remaining amine surface groups of gelatin with the ninhydrin assay. The

ninhydrin stock solution was prepared by dissolving 70 mg of ninhydrin in 20 mL of ethanol. Gelatin-G3.5 conjugates in very small amounts (1 or 2 mg) were dissolved in 1 mL of ethanol and further mixed with 1 mL of ninhydrin stock solution. The mixture solution was heated to 90 °C for 5 minutes after which it was cooled down to 25ºC. The concentration of amine groups ( [$NH_2$], mg/mL) was determined at the wavelength of 570 nm based on the calibration curve, which was established using pure gelatin of known concentrations. The standard curve for gelatin in ninhydrin is graphed in Figure 6.

**Figure 6**: Standard Calibration Curve of Gelatin (Ninhydrin Assay)




3.4.2 $^{1}$H-NMR Spectroscopy

$^{1}$H-NMR spectra of the synthesized polymers were recorded on a Varian superconducting Fourier-transform NMR spectrometer (Inova-400). Deuterium oxide ($D_2O$, 99.9%) was used as the solvent. The chemical shift for $D_2O$ is 4.8 ppm. Post-

processing was done using “Spin-Works” (free shareware) software courtesy of the University of Manitoba, Canada.

### 3.4.3 Mechanical Property Testing

Dog bone-shaped samples were cut (1.85 cm-length x 0.6 cm-outer diameter x 0.3 cm inner diameter) with a punch die for mechanical testing. The punch was positioned on the electrospun mat perpendicular to the mandrel’s axis of rotation. Six dogbones were cut from each mat and the sample thickness measured using digital calipers. Uniaxial tests were conducted using a Bionix 200 Mechanical Testing Systems with a 50N load cell. The offset edges of the dog bone specimen were loaded into the metal grips of the machine and tested at 10 mm/min until failure. Stress, strain, elastic modulus, peak load, and break were calculated and reported from the TestWorks4 computer software program.

### 3.4.4 Scanning Electron Microscopy (SEM)

Fiber morphology and fiber diameter were observed under scanning electron microscopy (SEM) employing a JEOL JSM-820 JE electron microscope with an acceleration voltage of 15kV. Samples were cut from each scaffold and gold sputter coated (Model 550; Electron Microscope Sciences) in preparation for scanning electron microscopy. An SEM photograph of each sample was taken at 1700x magnification. Thirty different fibers were measured using ImageTool 3.0 image visualization software (shareware provided by UTHSCSA). The software was calibrated using the micron scale bar of each photograph and average fiber diameter reported.

3.4.5 Porosity Measurement

Porosity, expressed as the percentage of void to total volume, was determined using the apparent volume method. The following equation was used to calculate porosity:

$$P = [1 - (\frac{V_g}{V_a})] \times 100 \qquad \text{Equation 1}$$

Where: $V_g$ = mass of the scaffold / density of collagen (1.41 g/cm$^3$)

$V_a$ = apparent volume of square section 1cm x 1cm x thickness

Sample thickness was measured using digital calipers. Three samples from each specimen were used to determine the porosity of each scaffold.

3.4.6 Permeability Measurements

The permeability of three punches (12 mm diameter) from each electrospun mat of each blend was measured using a meter developed by Scott Sell based on Ogston's theory of flow through systems of long thin rods.[46] Water was the fluid utilized for testing the samples. Permeability, represented as the Darcy constant ($\tau$), is calculated using the following equation:

$$\tau = \frac{Q\mu D}{tAp} \qquad \text{Equation 2}$$

where: Q=volume flowing through system, $\mu$=fluid viscosity (0.89 cP),

D=scaffold diameter (12 mm), A=cross-sectional area of scaffold ($\pi r^2$),

p=applied pressure (i.e. $\rho gh$= 1000 kg/m$^2$ * 9.8m/s$^2$* 1.5m(h); converted to atmospheres). t = time in seconds

Pore size was calculated by using the permeability determined in Equation 2 and the formula:

$$r = 0.5093 \times \sqrt{\tau}$$ Equation 3 [46]

3.4.7 Absorption and Water Swelling Behavior

The swelling ratio was used to evaluate the absorption properties of each dressing type. The swelling ratio of each test membrane was determined by immersing the sample in phosphate-buffered saline (PBS, pH 7.4) at room temperature. Subsequently, the weight of the swollen membrane was determined by sandwiching the membrane between two paper towels to remove excess water on the surface and weighed immediately. The swelling ratio ($R_{sw}$) of each test sample in PBS was calculated as follows:

$$R_{SW} = [\frac{(W_f - W_i)}{W_i}] \times 100$$ Equation 4

where: $W_f$ denotes the weight of the test sample after swelling and $W_i$ is its initial weight. Three samples per gelatin-polymer blend were evaluated to determine water swelling kinetics.

### 3.4.8 *In vitro* Degradation

The *in vitro* degradability of each test sample was assessed as a measure of physiologic degradation. The test samples were well immersed in PBS (pH 7.4) and incubated at 37°C for 24h. After degradation, the test samples were centrifuged for 20 min in deionized water, frozen, then lyophilized for 5 h and weighed. The ratio of weight loss ($R_{WL}$) due to *in vitro* degradation for each test sample was calculated using the formula:

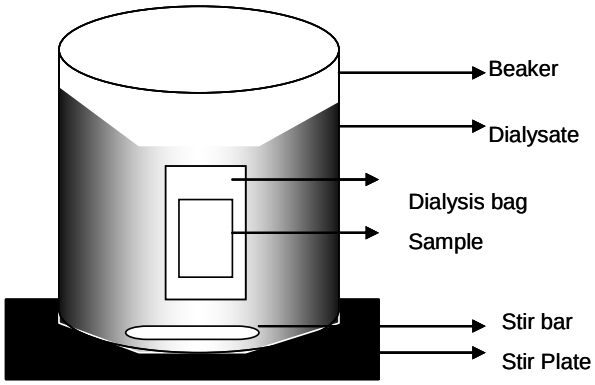
$$R_{WL} = [\frac{(W_i - W_f)}{W_i}] \times 100 \qquad \text{Equation 5}$$

where $W_f$ denotes the weight of the test sample after *in vitro* degradation and $W_i$ is its initial weight. Three samples per gelatin-polymer blend were evaluated for this analysis. Additionally, degradation was evaluated for 7 days by the above method; to evaluate degradation rate for each hybrid.

### 3.4.9 Drug Release Kinetics of Doxycycline

Drug release kinetics were determined through dialysis of the drug in DI water.

**Figure 7**: Analytical Set-up for Drug Release Kinetics



To study drug release kinetics, samples of each electrospun gelatin-polymer mat were obtained, weighed and loaded into a dialysis tube. After the tubing was clamped at one end, 20mL of PBS (pH 7.4) was added. At least 50% of the total scaffold weight was utilized and the remainder of the sample retained for replicate testing (to evaluate accuracy and reproducibility). The filtrate, 400mL of DI water, was added to three separate beakers and heated to 37 °C. After heating to the set-point, the sample (dialysis diskette) was immersed in each beaker. The 50/50 w/w gelatin-polymer blends were evaluated at the same time, but in 3 different beakers. The 70/30 w/w gelatin-polymer blends were tested in the same manner. To evaluate the short-term drug release, samples were collected at the following time intervals (in minutes): 2, 5, 7, 10, 15, 25, 35, 45, 60, 75, 90,120, 150, 180 and 240 for 15 samples. The absorbance value was recorded from the UV-Vis spectrometer for each sample per hybrid at the cut-off wavelength of 190 nm. After each measurement, the sample was re-injected into the beaker to keep the level of filtrate constant. The observed absorbance value was referenced against the standard curve of DC and the concentration of the drug was interpolated. Doxycycline was dissolved in DI water at a concentration of 1 μg/mL and scanned for the maximum absorbance on the UV-Vis spectrometer. Figure 8 shows that the maximum absorbance for DC is at a cut-off wavelength of 190 nm. The standard curve of DC was developed using a cut-off wavelength of 190 nm and is shown in Figure 9. The initial amount of DC was calculated from the ratio of the sample weight divided by total scaffold weight equal to unknown DC initial weight divided by total DC. The initial drug weight ranged from 2 to 5 mg per sample.

**Figure 8**: UV-Vis Scan of DC ( 1 μg/mL)

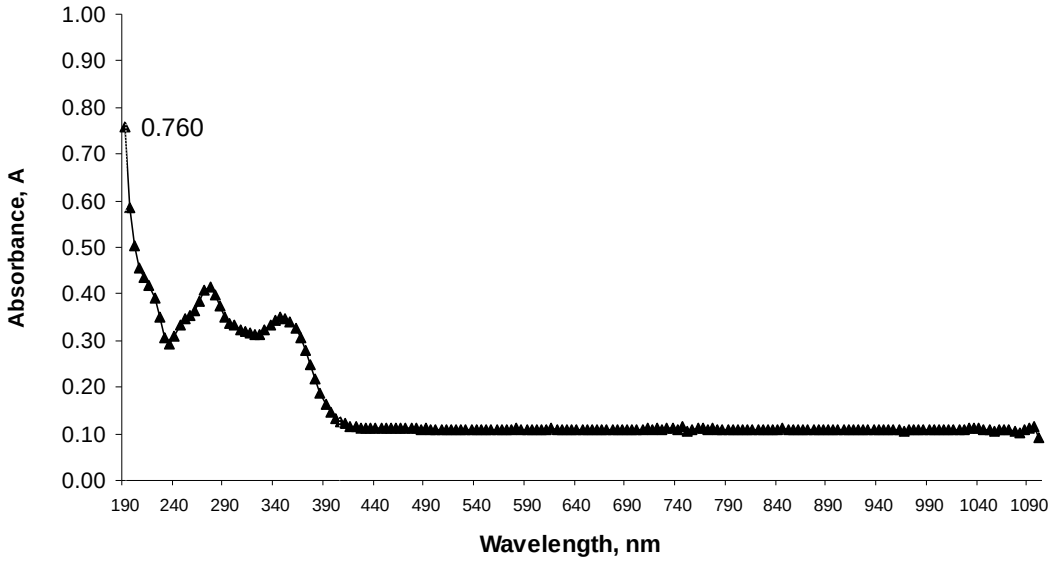
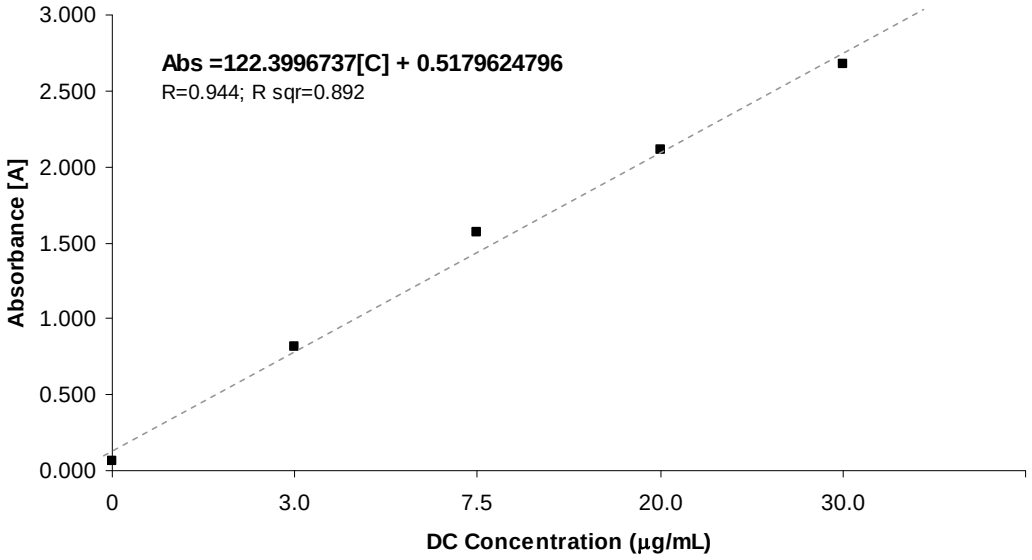



**Figure 9**: Standard Calibration Curve of DC (190 nm)



Drug release over time was determined by calculating percent drug release. The percentage of drug release was calculated as follows:

$$\%DR = (\frac{C_t}{C_i}) \times 100 \qquad \text{Equation 6}$$

where $C_t$= concentration of drug released at time (t), and $C_i$ = initial concentration of drug in the sample. Initial drug concentration was calculated from the ratio of specimen weight divided by total sample weight equal to the mass of DC divided by the total DC added to the electrospinning solution. Initial concentration of DC per test specimen ranged from 0.04 to 0.05 mg/mL.

### 3.4.10 Statistical Analyses and Graphical Representations

Minitab Statistical analysis software was utilized to perform Analysis of Variance (ANOVA), Analysis of Means (ANOM) and Tukey's Test for Significance to determine statistical differences (if any) between fiber diameter and the mechanical properties of peak stress, modulus and breaking strain across different blend compositions and w/w ratios. Analysis of variance is a statistical test that compares the variation within a group to the variation among groups. A positive ANOVA indicates that the differences among groups are more substantial than would be expected by chance alone. Analysis of means is a graphical analog used for testing the equality of population means. MINITAB displays a graph, similar to a control chart, highlighting group means that are different from the overall mean. If differences among the sample groups were found, Tukey's pair-wise comparisons were done to determine which values were significantly different. Tukey's

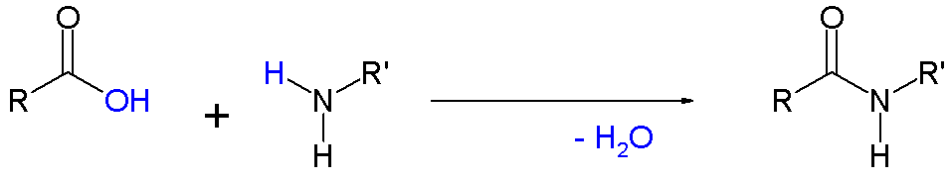
method compares the means for each pair of group levels using an overall error rate that is specified by the user. Results are presented as confidence intervals for the difference between pairs of means. If an interval does not contain zero, the means are statistically different, else accept the null hypothesis (means are the same). An alpha level of 0.05 was selected for all statistical tests. Graphical representations were constructed using Microsoft Excel 2003 and MINITAB.

# CHAPTER 4 Results and Discussion

## 4.1 Preparation and Characterization of G3.5-Gelatin Conjugates

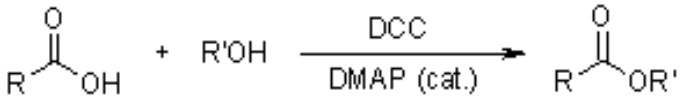
EDC chemistry was used to conjugate gelatin to PAMAM G3.5 dendrimer (Figure 10). A UV-Vis spectrophotometer scan of pure gelatin and PAMAM G3.5 yielded overlapping peaks; which negates this analytical tool from confirming the synthesis. Subsequently, verification of the conjugation of gelatin to G3.5 was indirectly determined by measuring the remaining amine surface groups of gelatin with the Ninhydrin assay. Ninhydrin reagent reacts with amino acids resulting in a purple colored solution.[47] The absorbance can be measured at the 570 nm wavelength on a UV-Vis spectrophotometer. Per the standard curve for gelatin with ninhydrin reagent (Figure 3), a 1 mg/mL sample of gelatin would have an absorbance value of approximately 0.1685. Ninhydrin reagent solution was added to a 1 mg/mL sample of the G3.5-gelatin conjugate; measured at 570 nm on the UV-Vis. The measured low absorbance value of 0.027 resulted from the decrease of the terminal amine groups $-NH_2$ of the gelatin due to the coupling reaction.

**Figure 10**: Schematic of Reaction to Form an Amide Bond [48]



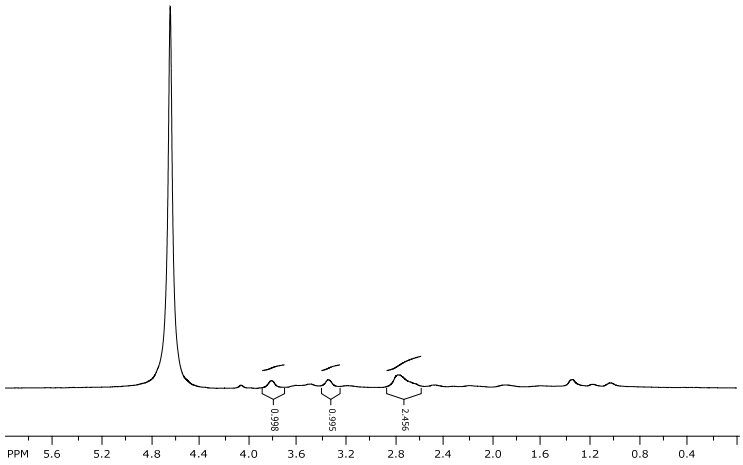
The Steglich esterification method of forming esters under mild conditions was attempted to conjugate PAMAM G3.5 dendrimer to doxycycline (Figure 11). Steglich esterification involves activating the carboxylic (-COOH) groups of the dendrimer with DCC. After activation, DMAP is used as a catalyst. We were not successful in conjugating DC to PAMAM G3.5. The hydroxyl groups of the DC were inaccessible; and could not be used to form an ester bond with the carboxyl groups of the dendrimer

**Figure 11**: Schematic of Steglich Esterification to Form an Ester Bond [49]




The conjugation of G3.5 to DC was not confirmed by $^{1}$H-NMR as shown in Figure 12 below. The peak for DC was not evident in the NMR image.

**Figure 12**: $^{1}$H -NMR of DC-PAMAM G3.5

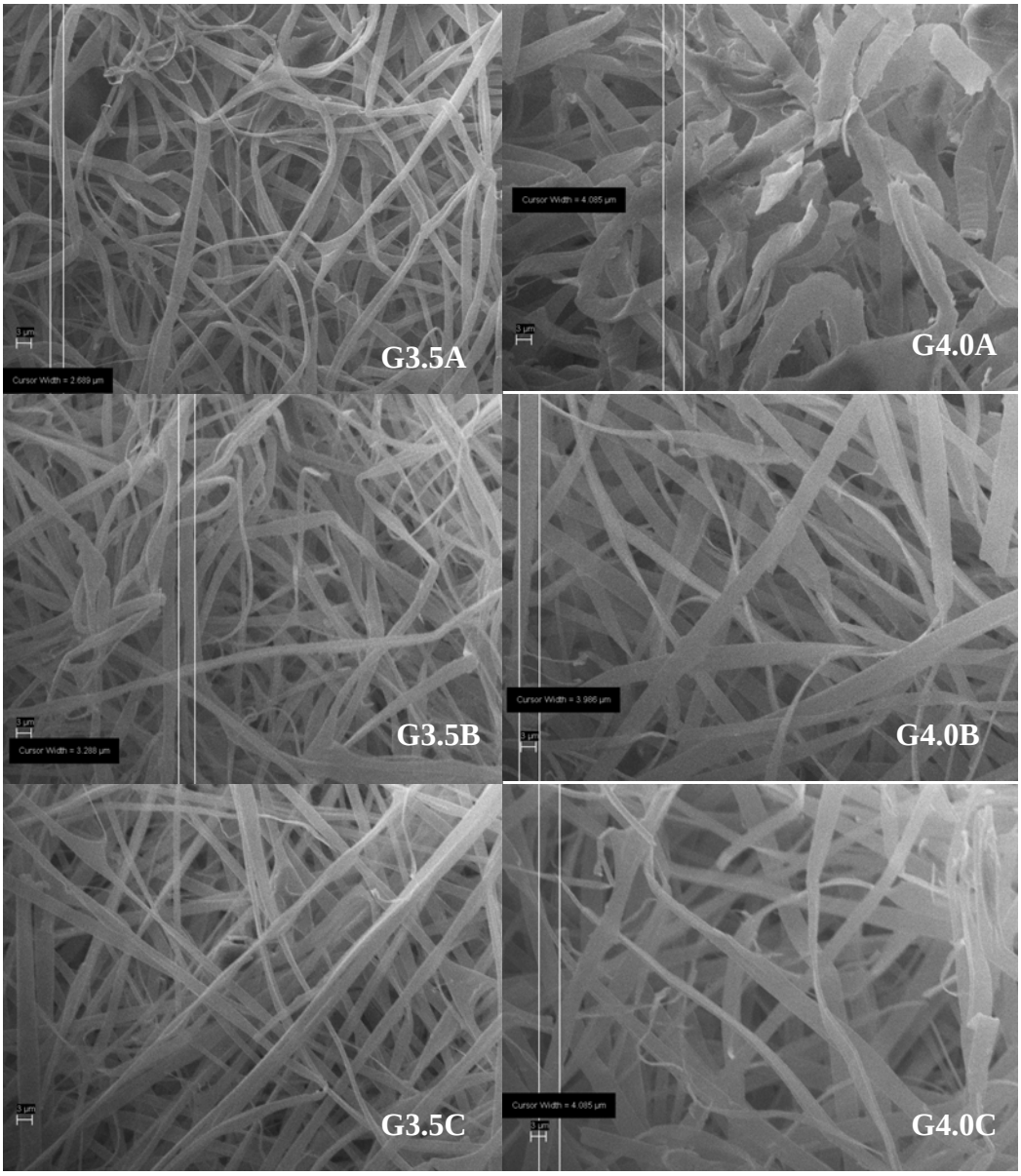



The yield of the G3.5-DC reaction synthesis ranged from 35 to 45% in five replicates of the reaction steps. Low yield can be attributed to product purification through dialysis.

The dialysis tubing used in the purification process had a molecular weight cut-off (MWCO) of 12000-14000. Doxycycline is small enough to escape through the pores. Due to the poor yield of this reaction and inadequate reproducibility, other therapeutic drugs are being explored in our laboratory. Because we were not successful in the synthesis of conjugating PAMAM G3.5 to DC, the drug release kinetics from the electrospun wound dressings will be performed using encapsulation of DC in the spinning solution.

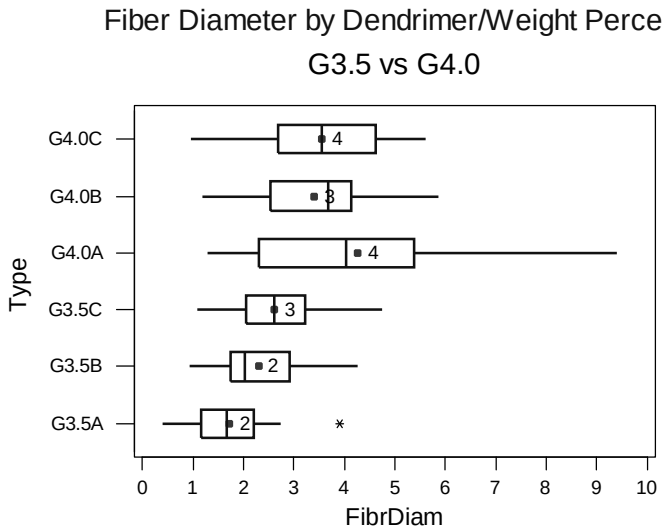
Two different PAMAM dendrimers, G3.5 and G4, were electrospun with gelatin to evaluate scaffold morphology and mechanical properties. Three different concentrations of dendrimer in gelatin were electrospun and evaluated. The G4 dendrimer/gelatin mats showed significantly larger fiber diameters, with means ranging from 3.4 to 4.2 μm. and displayed a flattened and oblong cross-section. The G3.5 dendrimer /gelatin mats had smaller diameter fibers, ranging from 1.8 to 2.5 μm on average. The G3.5 dendrimer/gelatin hybrids exhibited a cross-section similar to pure electrospun collagen and gelatin. The full generation PAMAM dendrimers have a positive charge; whereas the G3.5 dendrimers have a negative charge. These charge differences influence the electrical field during electrospinning, increasing the charge differential thereby altering the fiber diameter. It is believed that solvent evaporates more readily from the positively charged polymer solution, forming a skin layer on the exterior. After the fiber is formed, the outer skin collapses forming an elongated, oval cross-section. These data are shown in Figures 13 and 14. Electrospun scaffolds containing PAMAM G3.5 dendrimer had the desired morphology and fiber diameter to resemble native ECM and will be further evaluated.

**Figure 13**: SEM of PAMAM G3.5 and G4.0 Scaffolds




**G3.5A**:4mg, **G3.5B**:6mg, **G3.5C**:10 mg, **G4.0A**:4mg, **G4.0B**:6mg, **G4.0C**:10 mg, scale bar = 3 μm

**Figure 14**: Fiber Diameter of PAMAM G3.5 and G4.0 Scaffolds




Fiber Diameter measured in micrometers

An additional study was done to determine if DC drug encapsulation impacts spinnablity and fiber diameter. Conjugates of G3.5 and gelatin, along with pure gelatin solution containing DC were mixed in solution with TFE and electrospun. A comparison of means for strain, stress and Modulus are summarized in Table 9. Mechanical strength, as measured by stress and strain, is significantly different for the scaffold mats of G3.5-gelatin conjugates (at 0.10 MPa, 0.16 mm/mm) versus pure gelatin (at 1.40 MPa, 0.06 mm/mm) or gelatin with encapsulated DC (at 0.96 MPa, 0.06 mm/mm). The encapsulation of DC into the scaffold did not significantly impact mechanical properties. The statistical difference of significantly lower stress in the scaffolds with G3.5-gelatin conjugates is attributed to the smaller diameter fibers.[50] Gelatin scaffolds are easily dissolved in aqueous environments. Synthetic polymers were added to increase mechanical strength and reduce dissolution rates; and further characterize scaffolds.

**Table 9**: Mechanical Properties of Electrospun Gelatin

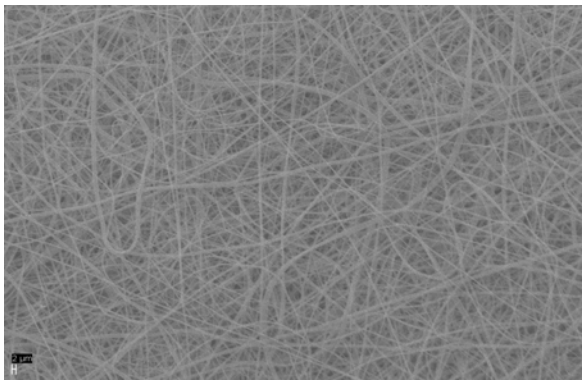
| Scaffold | Strain (mm/mm) | Stress, (MPa) | Modulus, MPa |
|---|---|---|---|
| Gelatin | 0.06 ± 0.01 | 1.40±0.58 | 37.59 ± 16.09 |
| Gelatin+DC | 0.06 ± 0.02 | 0.96±0.52 | 26.09 ± 10.44 |
| Gelatin-G3.5 | 0.16 ± 0.04* | 0.10±0.02* | 1.13 ± 0.21* |

Comparison of Means – Means with an asterisk (*) are statistically different
( $\alpha$=0.05, Pr < 0.001)

**4.2** Gelatin/dendrimer Hybrid Morphology

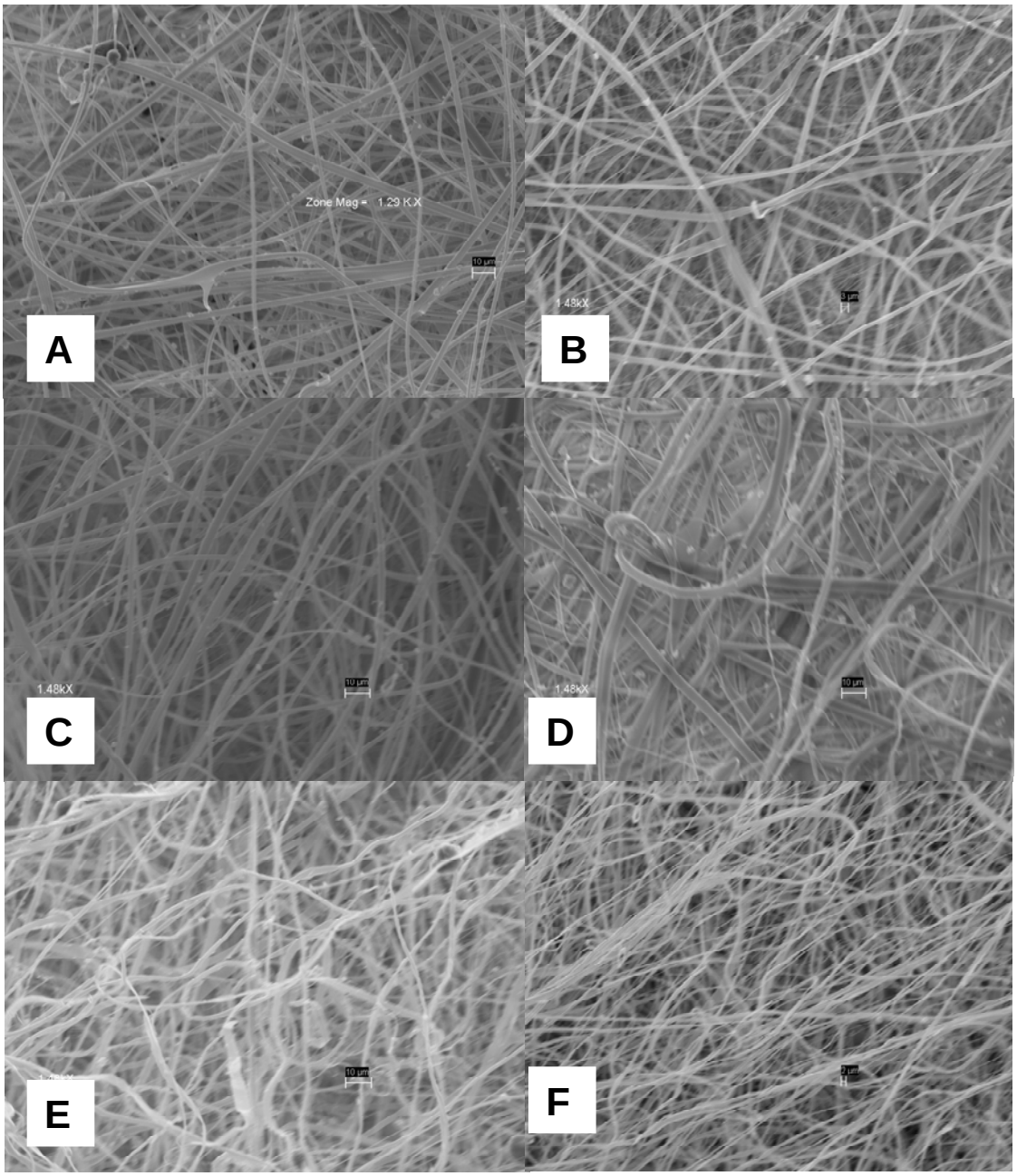
The SEM photographs were similar for all of the polymer gelatin blends; and similar to electrospun gelatin as shown in Figure 15. Gelatin and the synthetic polymers were successfully electrospun into uniformly distributed fibrous scaffolds (Figure 16). All samples displayed homogenous fibrous structures when observed via SEM.

**Figure 15**: SEM of Electrospun Gelatin



Scale bar: 2 μm, 1.05 kX magnification

**Figure 16**: SEM Micrographs of Polymer Blends




**A**:50/50 PCL/gel,**B**:70/30 PCL/gel,**C**:50/50 PGA/gel,**D**:70/30 PGAgel,**E**:50/50 PLGAgel,**F**:70/30 PLGA gel

Scale bars: A=10 μm, B=3 μm, C=10 μm, D=10 μm, E=10 μm, F=2 μm

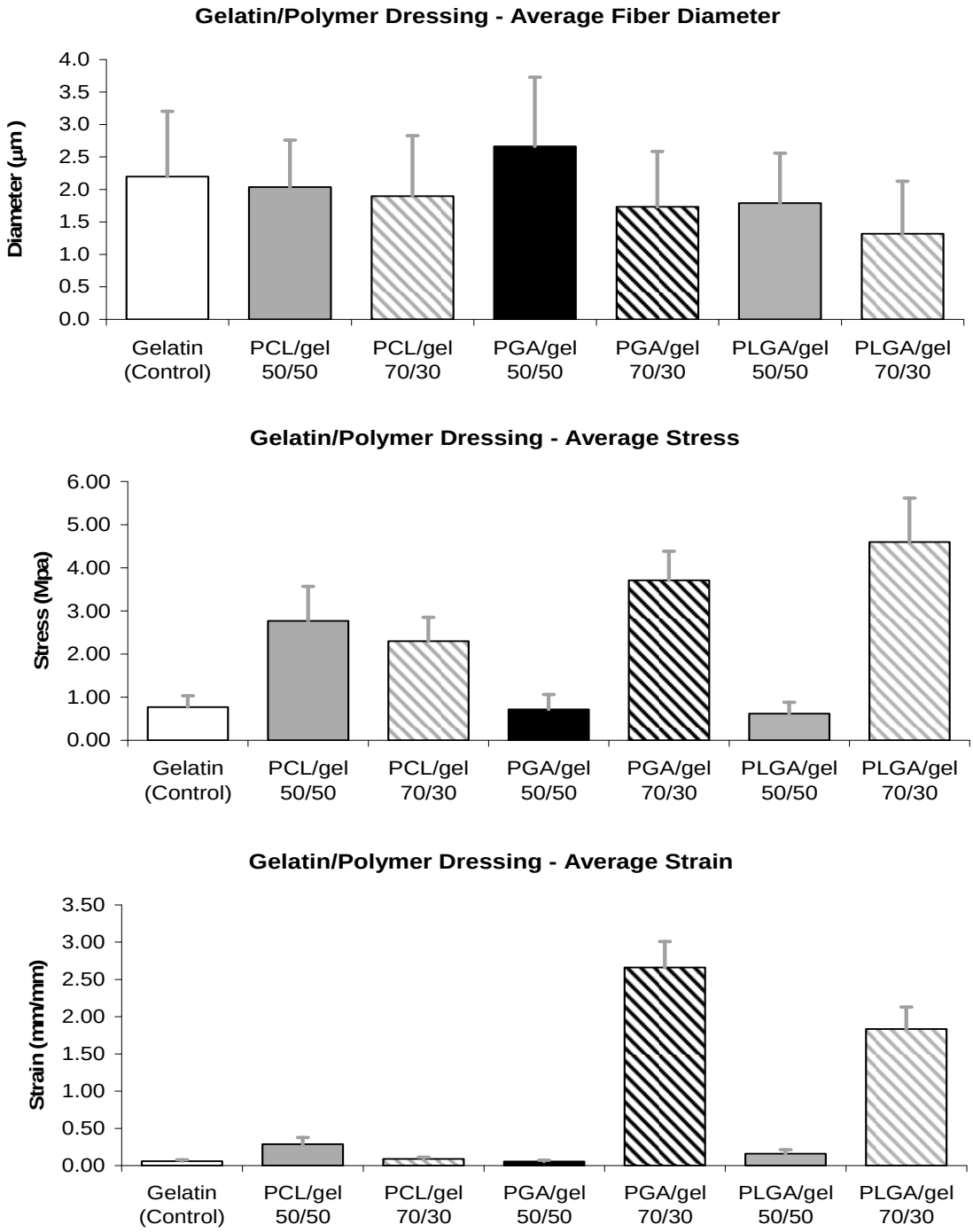
The scaffold morphology, represented by fiber and pore diameter and visual observations, is similar to previously reported data of electrospun gelatin scaffolds.[18,44] The fiber diameter and mechanical properties are summarized in Table 10 and graphically represented in Figure 17. Fiber diameters varied with the varying w/w ratio of gelatin to polymer and polymer type. Much of the previously published electrospun data on gelatin show that increasing gelatin concentration increases fiber diameter. Additionally, increasing fiber diameter causes an increase in mechanical strength. [50]

**Table 10**: Summary of Mechanical Properties.

| | Fiber Diameter ( μm) | Stress (MPa) | Modulus (MPa) | Strain (mm/mm) | Break Energy (N-mm) |
|---|---|---|---|---|---|
| Gelatin (Control) | 2.2 ± 0.1 | 1.6 ± 0.3 | 22.1 ± 7.3 | 0.06 ± 0.02 | 0.11 ± 0.04 |
| PCL/gel 50/50 | 2.0 ± 0.7 | 2.8 ± 0.8 | 41.9 ± 9.3 | 0.09 ± 0.02 | 0.32 ± 0.07 |
| PCL/gel 70/30 | 1.9 ± 0.9 | 2.3 ± 0.6 | 19.0 ± 5.2 | 0.39 ± 0.09 | 1.06 ± 0.54 |
| PGA/gel 50/50 | 1.7 ± 0.9 | *0.7± 0.3 | 18.2 ± 9.7 | 0.06 ± 0.01 | 0.13 ± 0.07 |
| PGA/gel 70/30 | *2.7 ± 1.1 | *3.7± 0.7 | 36.8 ± 13.9 | *2.66 ± 0.35 | *39.17 ±0.95 |
| PLGA/gel 50/50 | 1.8 ± 0.8 | 0.6 ± 0.3 | 9.2 ± 6.4 | 0.16 ± 0.05 | 0.15 ± 0.05 |
| PLGA/gel 70/30 | *1.3 ± 0.8 | *4.6 ± 1.0 | *47.5 ± 13.8 | *1.84± 0.30 | *43.62 ±0.38 |

*Statistically different means are marked (*)*

**Figure 17**: Graphical Summary of Fiber Diameter, Stress, and Strain



In this study, the gelatin concentration and synthetic polymer concentration were varied together. Therefore, determining the impact of changes in gelatin concentration on the mechanical properties was confounded with the changes in synthetic polymer concentration and type.

Fiber diameter of electrospun gelatin has been shown to be a function of solution concentration as concluded in our previous work. With the exception of the PGA/gel 70/30 blend (significantly larger fiber diameter) and PLGA/gel 70/30 blend (significantly smaller), fiber diameter was not statistically different. Additionally, the inclusion of DC in the spin solution had no effect on pore area or fiber diameter when comparing the mat to 100% gelatin as the control. The mean fiber diameter ranged from 1.3 um to 2.7 um with a pooled standard deviation of 0.9 (Appendix A). Statistical differences were analyzed using ANOVA in Minitab. The fiber diameters for PGA/gel 50/50 blend were significantly smaller than the PGA 70/30 blend; however, the opposite was true in comparing PLGA/gel 50/50 and 70/30 w/w preparations. The fiber diameter for the latter was significantly smaller. Possible contributors are the change in synthetic polymer weight affecting the spin solution viscosity; thus impacting fiber diameter.

The ANOVA and Tukey's comparisons confirmed graphical observations of stress by type and w/w blend ratios. Mean stress is statistically higher in the 70/30 PLGA/gel mat, which is significantly higher than the 70/30 PGA/gel blend; which is statistically higher than all other blends including the gel control. The larger diameter fibers in the PGA/gel 70/30 blend is the main contributor the higher stress; as larger diameter fibers are

stronger in tension. All variables of the fabrication process were controlled (i.e. the distance to mandrel, spinning voltage, mandrel rotation and geometry were held constant). Therefore the changes in fiber diameter and mechanical properties are solely due to the polymer type and weight ratios of gel to polymer.

The mean strain for the study mats ranged from 0.05 to 2.66 mm/mm with a pooled standard deviation of 0.18 (Appendix). The strain for PGA/gel 70/30 and PLGA 70/30 blends were significantly different from the strains exhibited in the other blends, including the electrospun gel control, and each other. This may be large due to similar physical and chemical properties of PGA and PLGA.

Regardless of the mechanical property measured, the greater the percentage of synthetic polymers PGA and PLGA in the blend, the more extreme the values for stress and strain. Both PGA and PLGA at the higher weight ratios showed greater mechanical strength, as measured by stress and break energy when compared to pure electrospun gel as the control. This infers that the addition of PGA and PGLA will yield stronger electrospun mats. One of the main reasons for blending and electrospinning the synthetic polymer with gelatin was to increase the mechanical strength and aqueous stability of the dressing. The encapsulation of dendrimer and DC in the spinning solutions did not significantly impact the stress and strain properties of the scaffold.

### 4.3 Porosity, Permeability and Swelling Behavior

The electrospun polymer matrices were evaluated for fluid and gas flow capability to confirm their viability as a wound dressing. The data is summarized in Table 9 and

graphically represented in Figures 18 through 21. Fluid and gas exchange is essential to ensure adequate wound healing. Cell migration, oxygen and moisture are essential to ensure tissue regeneration in chronic wounds. The ultimate test to determine the success of the proposed dressing is dependent on its ability to mimic native ECM and allow cells to infiltrate the construct, migrate through its thickness and proliferate as ECM is essential to remodeling.[51] Tissue regeneration is also facilitated by adequate nutrient flow, gas exchange and maintenance of a moist wound bed for proper healing. [52] Scaffold pore diameter, porosity, permeability and swelling and degradation behavior were evaluated as a means to measure its suitability and ability to ensure adequate flow of oxygen into the wound bed; while simultaneously maintaining a moist wound bed.

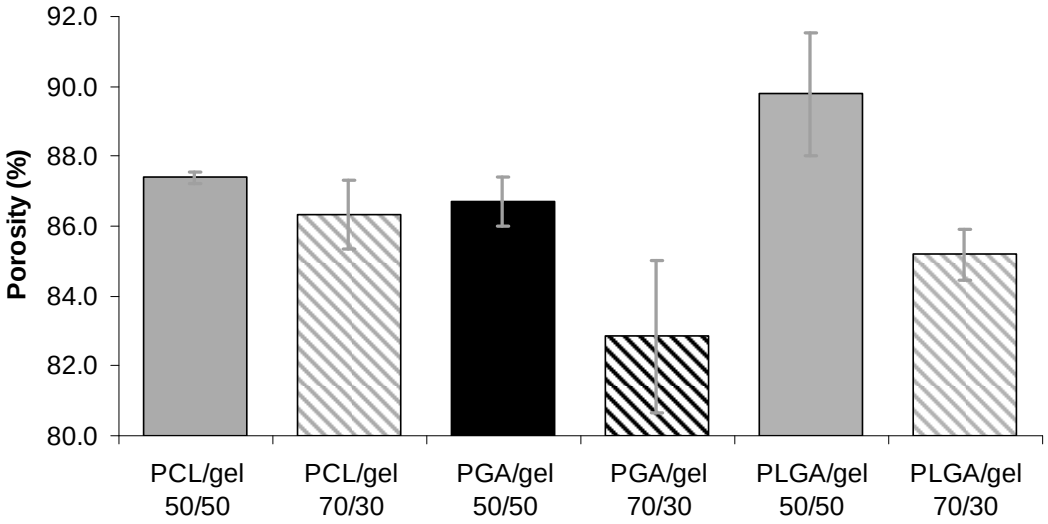
**Table 9**: Summary of Gel-Polymer Matrix Fluid Flow Capability

| | Pore Diameter ( μm)* | Porosity (%) | Permeability (D) | $R_{Swelling}$ (%) | $R_{Degradation}$ (%) |
|---|---|---|---|---|---|
| PCL/gel 50/50 | 0.28 ± 0.01 | 87.4 ± 0.2 | 0.0028 ± 0.0003 | 345.6 ± 10.7 | 28.3 ± 8.3 |
| PCL/gel 70/30 | 0.25 ± 0.11 | 86.3 ± 1.0 | 0.0024 ± 0.0015 | 296.3 ± 8.6 | 10.6 ± 1.2 |
| PGA/gel 50/50 | 0.31 ± 0.85 | 86.7 ± 0.7 | 0.0036 ± 0.0014 | 520.3 ± 41.3 | 30.4 ± 14.6 |
| PGA/gel 70/30 | 0.23 ± 0.04 | 82.9 ± 2.2 | 0.0024 ± 0.0008 | 294.6 ± 41.7 | 45.0 ± 7.1 |
| PLGA/gel 50/50 | 0.39 ± 0.02 | 89.8 ± 1.8 | 0.0048 ± 0.0021 | 347.1 ± 34.9 | 10.0 ± 9.3 |
| PLGA/gel 70/30 | 0.23 ± 0.39 | 85.2 ± 0.7 | 0.0024 ± 0.0007 | 256.7 ± 7.1 | 15.5 ± .2 |

Pore Diameter calculated from Equation 6 using permeability(*)

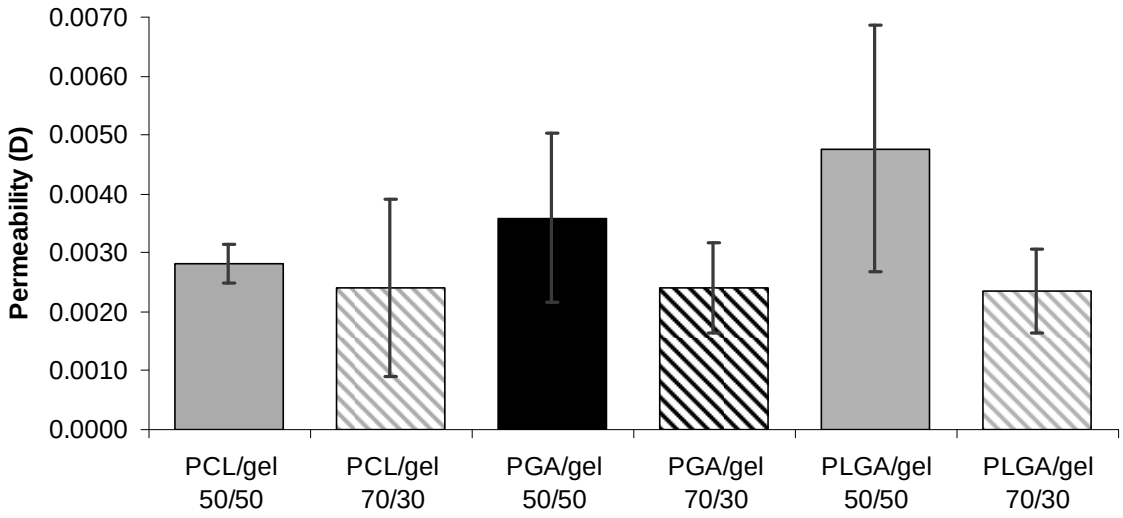
Porosity is a measure of the amount of void space in the electrospun scaffold and values are shown in Figure 18. The porosity ranged from 83 to 89%. The lowest porosity value was supported by SEM depictions of the 70/30 PGA/gel polymer blend. The scan shows minimal pores; which is also confirmed by pore diameter as calculated by the Darcy permeability. None-the- less, these levels are suitable as a TE construct as they are in the acceptable 70-90% range for adequate gas and nutrient exchange to underlying cells.

**Figure 18**: Gel/Polymer Dressing - Porosity




Permeability is a measure of the ease of flow through a scaffold. The permeability ranged from 0.0024 to 0.0048 Darcy. The lowest porosity value was supported by SEM micrograph of the minimal pore diameter. Permeability trended with porosity, in that the 50/50 PLGA/gel mat was the most porous and showed significantly higher permeability. Permeability measurements are depicted in Figure 19.

**Figure 19**: Gel/Polymer Dressing - Permeability




Water swelling results are presented in Figures 20 and 21. The swelling rate was found to be a function of polymer type and weight ratio of each individual component of the gelatin/polymer hybrids. For all the gel-polymer blends, the swelling rate showed a maximum value at the 30 minute time interval; thereafter reaching a plateau.

The 50/50 PLGA/gel mat showed the highest porosity (openness) and the highest permeability (ease of flow), as the two are interrelated. As expected, the least amount of swelling of the fibers occurred in this mat as a result of the increased void space, where fibers are non-existent. Likewise, the 70/30 PLGA/gel mat was one of the least porous structures and thus had the highest swelling rate for the two hour evaluation. The swelling ratios were higher for the 50/50 w/w versus 70/30 w/w blends, which is a result of the swelling behavior of the gelatin. These data are shown in Figures 18 and 19.

**Figure 20**: Gel/Polymer Dressing – Swelling Rate

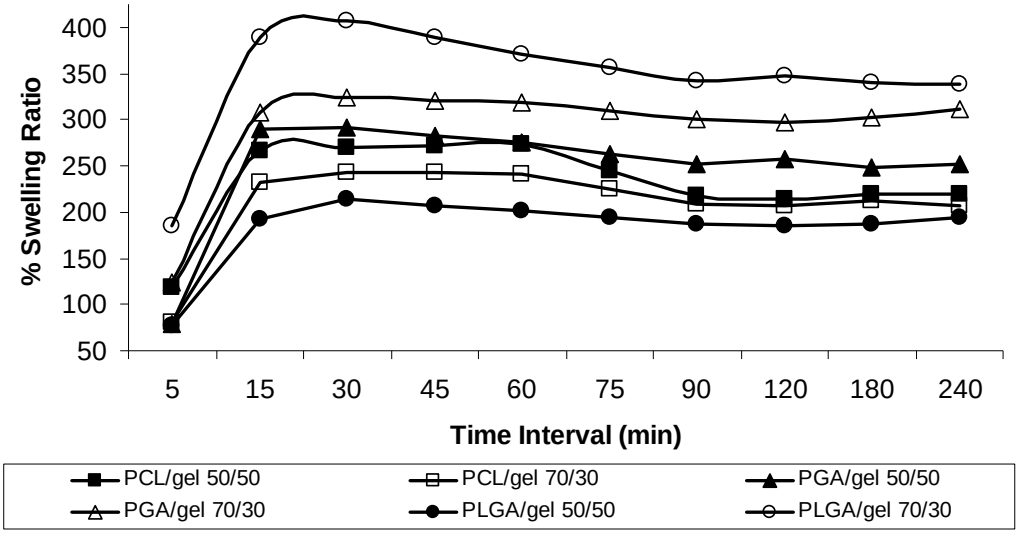
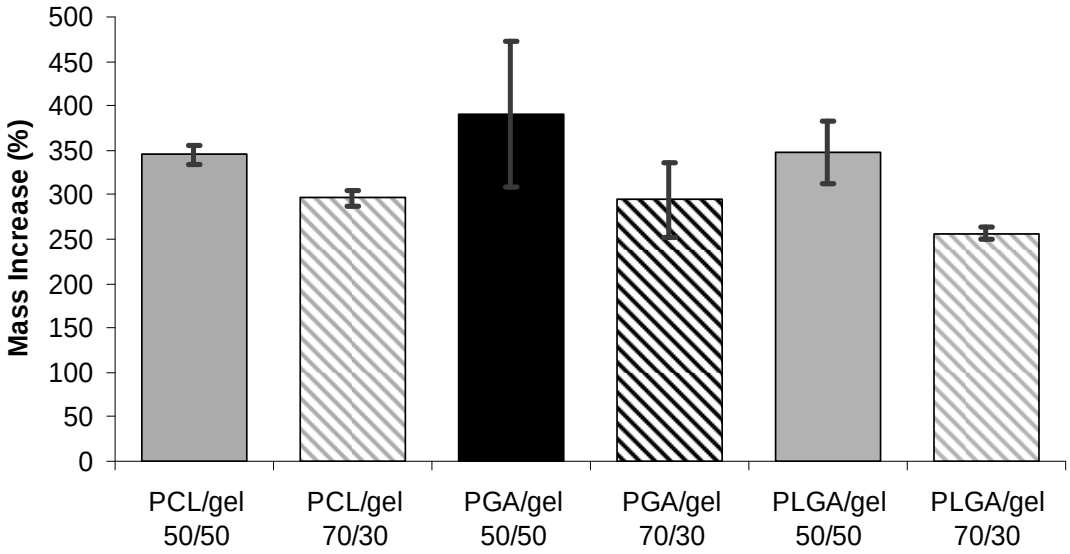
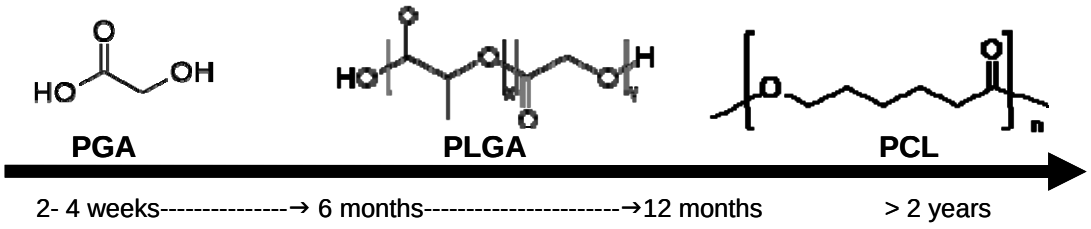



**Figure 21**: Gel/Polymer Dressing – Swelling Behavior



The synthetic polymers used in this study, PCL, PGA and PLGA, are susceptible to hydrolysis at various rates. The rate and extent of hydrolysis is heavily dependent on the hydrophilicity of the individual polymer and any co-polymers. The more hydrophilic the polymer, the more aggressive the hydrolysis will be. [53] The blending of the synthetic polymers with gelatin as a co-polymer impacts the hydrophilicity of the electrospun polymer matrix and their electrospinning conditions since intrinsic viscosity changes. Subsequently, the degradation rates will also be impacted. Based on the physical and chemical properties of the synthetic polymers evaluated in this study, PGA should have the faster rate of swelling, as it is the most hydrophilic of the three. Figure 22 is a depiction of the bulk degradation sequence for the synthetic polymers used in this study.

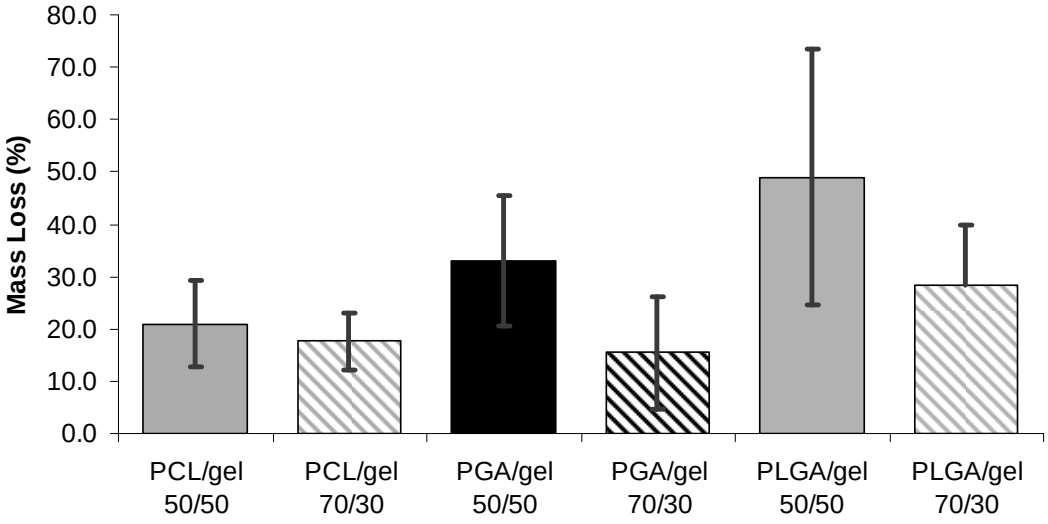
**Figure 22**: Theoretical Bulk Degradation Sequence




In our evaluation, the 50/50 gelatin/polymer blends degraded at a much faster rate. This is mostly due to the higher amount of gelatin; thus faster hydrolysis in comparison to the higher amouNt of synthetic polymer. The synthetic polymer blends with PCL showed the least amount of degradation, as PCL can take up to 2 years to degrade in-vivo. Regardless of the blend ratio, PLGA degraded at a significantly higher rate than the other two polymers (PCL and PGA); which did not follow the theoretical prediction. The 24-hour

degradation is shown in Figure 23. Note that the scaffold with the highest permeability and porosity, showed the fastest degradation.

**Figure 23**: Gel/Polymer Dressing – Degradation




The degradation results were not consistent with the water swelling results, but were consistent with the observed trend for permeability. Bulk degradation, considered homogenous hydrolysis, occurs when the diffusion of water into the polymer matrix is faster than the overall rate of hydrolysis.[54] As water diffuses into the matrix and cleaves the ester bonds, erosion of the fibers occurs leading to a decrease in mass. For PLGA, the degradation time is dependent on the monomer ratio of lactide to glycolide. The more glycolide monomers, the more hydrophilic the co-polymer. PLGA 85:15 is less hydrophilic than PGA and should have degraded at a slower rate. However, the higher porosity and permeability in the PLGA/gelatin 50/50 w/w electrospun mat is credited as a major contributor to the faster degradation, since degradation rate is also dependent on the

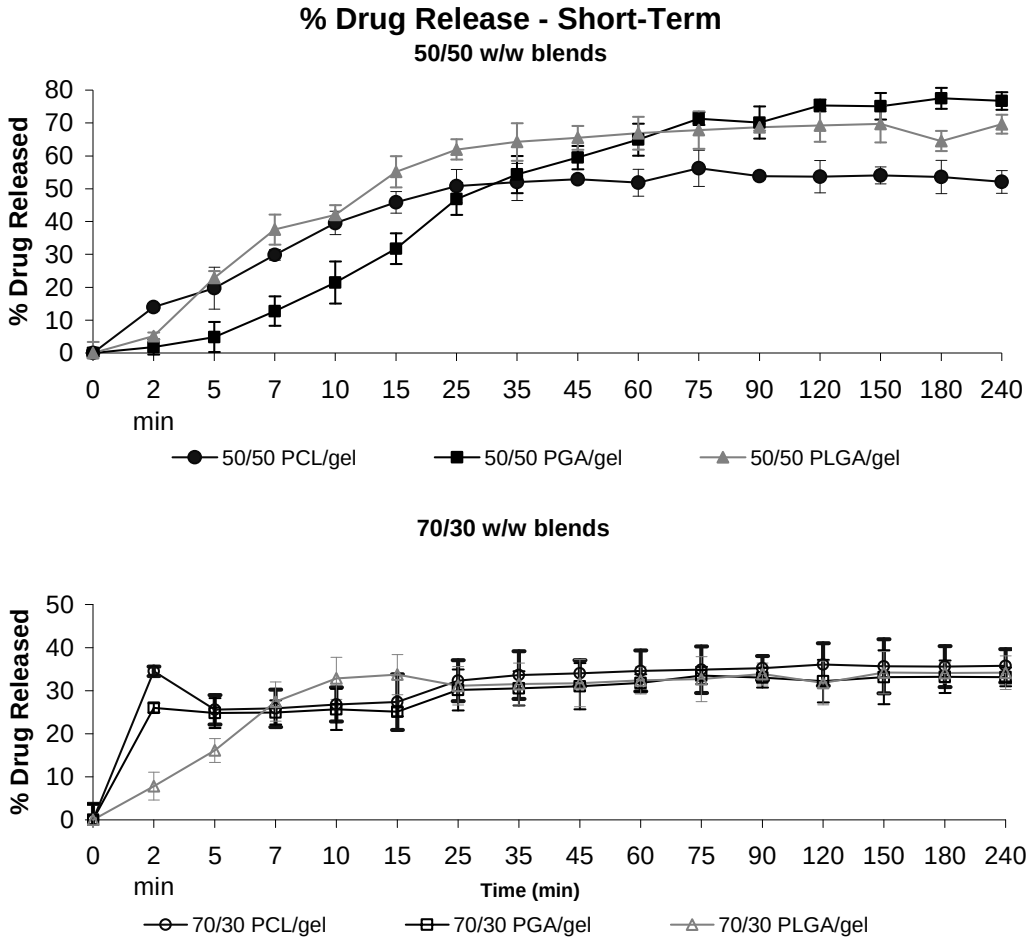
morphology, porosity and permeability. Degree of crystallinity also affects degradation. The greater the degree of crystallinity, the slower the degradation. Poly (lactic-co-glycolic) acid is amorphous which results in a faster *in vitro* degradation rate.

4.4 Drug Release Kinetics

It has been shown that the release of active drugs as function of time can be measured by means of diffusion of the drug from a porous membrane. Diffusion controlled drug release was achieved by using dialysis tubing loading with the sample, containing the drug and PBS as the dialysate. Doxycycline was measured when the drug diffused from higher concentration (inside the porous membrane) to the filtrate, which in our study was deionized water. Figure 24 is a graphical representation of the drug release of DC over time. We observed that an increase in the weight of the synthetic polymer resulted in the lower amount of released drug. In both ratios, regardless of polymer type, controlled release of the drug is apparent. The release, however is short-lived, reaching a plateau within two hours, with no significant release thereafter. We hypothesize that the plateau is due to hydration of the gelatin and synthetic polymers causing the fibers to swell, thus increasing their fiber diameters and subsequently reducing pore size. The reduction in pore size traps the drug, sequestering it inside the interior of the mat. Extending the drug release studies for long-term evaluation (days to weeks) is required to confirm our supposition that as the mat swells to its maximum size; the drug release kinetics will plateau; and then increase as the PGA, PCL and PLGA continue to hydrolyze

thus releasing more of the drug. This degradation will lead to additional release of the entrapped drug (i.e. residual drug).

**Figure 24**: Drug Release Kinetics (Synthetic Polymer/Gelatin blends)




Gelatin is soluble in water; whereas the synthetic polymers used in this study have no to minimal solubility in water. The burst in drug release is attributed to the surface degradation and dissolution of the gelatin. The higher release rate of the 50/50 w/w blends

in comparison to the 70/30 w/w blends is mainly due to the higher amount of gelatin; therefore faster degradation (i.e. hydrolysis) of the matrix. As the gelatin dissolves, the drug is released from the interior of the mat. The drug release profile for the 50/50 w/w blends closely followed the degradation rate profile. As noted previously, PGA had more mass loss in 24 hours and over a 7 day time-frame. The drug release of DC was also higher for the 50/50 w/w PGA/gelatin blend. More than 50% of the drug was released; which may indicate that some of the polymer, however minimal, hydrolyzed as well. An alternative means of evaluation, for example High Performance Liquid Chromatography (HPLC), should be employed in lieu of measuring the absorbance to determine the concentration of the released drug. High Performance Liquid Chromatography will allow more accurate measurements of drug concentration as it can better partition different components of the blend based on their affinity. [55-56] An article by S.M. Sunaric, et al., confirms that the peak absorbance for DC is measured at approximately 270 nm and 360 nm. [57] As indicated in Figure 8, two secondary peaks occur at approximately 270 nm and 360 nm; however a wavelength of 190 nm was used to measure released drug (DC) since the maximum absorbance was observed at this point. Sunaric's research evaluated physiologic degradation of DC in samples with concentrations of DC ranging from 2.97 to 17.78 μg/mL. We used UV-Vis to measure concentrations of DC within the same range. According to British Pharmacopoeia, the official method for DC determination is liquid chromatography with UV detection. This further confirms the necessity of our future work to develop an HPLC method for analysis of DC released from our polymer scaffolds.

## 4.5 Conclusions

Our work demonstrated that gelatin/dendrimer hybrids could be formed using full and half generation dendrimers and electrospun with stabilizing synthetic polymers and the antibiotic Doxycycline. The half generation dendrimer produces significantly smaller fibers that resemble electrospun collagen and gelatin (fabricated using the same operating parameters and conditions). Three different stabilizing polymers were electrospun with gelatin and PAMAM G3.5 dendrimer to form the hybrids. Synthetic polymer type and weight ratio was shown to affect the permeability, porosity, mechanical properties and water swelling and degradation of the electrospun mats.

The increased weight addition of PCL, PGA and PLGA to the gelatin/dendrimer hybrids decreased the porosity and permeability of the electrospun mat; corresponding to decreased fiber diameters and pore sizes. Theoretically, decreased fiber diameter should result in decreased mechanical stress, however, the stress values for the synthetic polymers far exceeds that of gelatin. Subsequently, the greater the amount of synthetic polymer, the higher the stress and strain values of mats. As proven in statistical analyses and graphical representations, the greater the weight ratio of synthetic polymer, the higher stress and strain levels the fibers could withstand.

Poly(glycolic acid) is more hydrophilic than PCL and PLGA. As such, the observed degradation and swelling ratio of the PGA containing mats versus the other two was at a much faster rate. This faster degradation resulted in a higher release of the drug in the time-frame analyzed. Drug release kinetics for all compositions showed a controlled

release over a four hour time frame. A statistical evaluation of the drug release profile for each polymer mat was not performed.

The success of fabricating an electrospun gelatin/dendrimer hybrid that can be utilized as a wound dressing with targeted and controlled drug release was demonstrated and achieved in this study. The synthetic polymer type and amount in the gelatin/dendrimer hybrids can be varied to influence the morphology, mechanical properties, swelling behavior and degradation, thus impacting its drug release kinetics.

# CHAPTER 5 Summary and Future Work

## 5.1 Summary

In the past few years, significant effort has been devoted to researching and developing nanomedicine and nanotechnology for controlled and targeted drug delivery. Presently, biomaterials and drug development has shown an increased focus on formulating therapeutic agents in biocompatible nanocomposites, like liposomes, micelle systems, nanocapsules and polymer conjugates, that are sub-micron sized. Advances in localized and tissue-specific delivery of small particles, like anticancer drugs, gene vectors, antimicrobials and other therapeutic agents, improve drug efficacy and decrease toxicity, and reduce drug side-effects. [58-59] The utilization of dendrimers as nano-sized drug carriers is expanding. Dendrimer drug carriers can be easily tailored to deliver both hydrophilic and hydrophobic drugs effectively and efficiently. Dendrimers exhibit surface charges that can be exploited to control pH and temperature sensitivity, as well as electrospun product characteristics. However, the research in developing gelatin/dendrimer hybrids for use as a wound dressing is limited.

Gelatin must usually be electrospun and cross-linked to acquire the mechanical properties and suitable characteristics as a TE scaffold. [60] In this study, gelatin/dendrimer and synthetic polymer matrices were formed by electrospinning and characterized to quantify their physical morphology, gas and nutrient flow capability, as well as swelling behavior, degradation rate and drug release kinetics. The gelatin/dendrimer hybrids that were fabricated show promise as a bioactive dressing to treat chronic wounds. They are

highly adaptable, and exhibit the properties necessary to maintain a moist wound bed to promote wound healing. Additionally, the controlled and targeted release of Doxycycline has the potential to facilitate wound healing by inhibiting MMPs that degrade the reconstituted ECM.

The polymer matrix can be engineered to control wound bed moisture by altering synthetic polymer type and weight ratio. Leveraging the amount of gelatin allows better swelling behavior and moisture retention. Wound bed moisture is critical to promote wound healing and ensure adequate nutrient and gas exchange, as well as cell migration, within the wound. The electrospun mat also mimics the native ECM, which theoretically, should foster cell adhesion, migration and subsequently proliferation. The attractive features and easy adaptability of the gelatin/dendrimer hybrid developed in this study will enable its application as a bioactive dressing to promote wound healing.

## 5.2 Future Work

This study attempted to characterize the physical properties, swelling, degradation and drug release profiles of gelatin/dendrimer hybrids for biomedical applications as a wound dressing. Doxycycline has been shown to inhibit MMPs, which are at excessive levels in chronic ulcers. However, this activity was not evaluated in this study. Studies designed to evaluate the impact of DC, released to the wound bed, will be conducted in our future work. Assays to measure the level of degradative enzyme contained in wounds covered by the proposed gelatin/hybrid and those using a control will be evaluated. *In*

*vitro* studies of the dressing's ability to provide a bacteria-free environment to facilitate wound healing will also be conducted.

The wound healing process is complex and involves interactions of various cells and matrix components to create a provisional matrix, a platform by which cells can migrate and adhere to, leading to eventual closure of the wound. Wound closure, i.e. re-epithelialization, involves the controlled migration of fibroblasts and keratinocytes. Recent research has shown that the inclusion of basic fibroblast growth factor (FGF) and transforming growth factor beta (TGF-β) accelerates cell growth and wound healing. [61] Human skin equivalents have been utilized to assess the acceleration of wound healing and the impact of additives like growth factors and proteins like collagen in wound dressings. In a study by Schneider and Wang, the rate of wound contraction was successfully measured and accelerated by the inclusion of epidermal growth factor (EGF) in electrospun silk fibroin scaffolds. [62] Cell interaction and proliferation studies were not conducted to assess the bioactivity of the dressing and its ability to facilitate or accelerate wound healing. Expanding this project to include *in vitro* and *in vivo* studies evaluating the migration and proliferation of fibroblasts and keratinocytes is a necessary milestone in our future work.

Chronic wounds including diabetic foot ulcers, pressure ulcers, and venous leg ulcers have been shown to have abundant biofilm communities. In a study by James, et al., chronic and acute wounds were evaluated for biofilms and the bacterial microorganisms inhabiting these wounds were characterized. [63] Sixty-percent (60%) of the chronic wound specimens that were evaluated by this group contained biofilms, compared to only 6% of

the acute wound specimens. Further, molecular analyses of chronic wound specimens examined in their evaluation revealed diverse polymicrobial communities and the presence of bacteria, including strictly anaerobic bacteria, not revealed by culture.

Bacterial biofilm prevalence in chronic wounds adds further complexity in trying to accelerate wound healing. Very few antibiotics have been shown to effectively mitigate or eradicate biofilms. [64-65] Bacterial vaginosis and urinary tract infections show significant biofilm formation, which contributes to a high rate of recurrence and multiple treatments to effect resolution. The antimicrobials, fleroxacin, ampicillin and gentamicin were found to effectively eliminate a lactose-negative, streptomycin-resistant uropathogenic strain of Escherichia coli in a study conducted by Morck, et al. [66] Fleroxacin eliminated E. coli from the catheter surfaces and from tissues adjacent to the catheter. The ability of fleroxacin to effectively eliminate biofilm bacteria from catheter surfaces and adjacent tissues shows promise as a potential drug for inclusion in the gelatin/dendrimer hybrid to treat chronic wounds.

We have successfully fabricated and characterized gelatin/dendrimer hybrids for use as a potential wound dressing. Our future work focuses on the cell interaction and bioactivity of the gelatin/dendrimer hybrids. Gentamicin, doxycycline and fleroxacin will be encapsulated and/or conjugated to the gelatin/dendrimer hybrids and their efficacy in mitigating biofilm formation in chronic wounds is also a major focus in our future work.

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# Appendix

### One-way ANOVA: Stress versus Blend

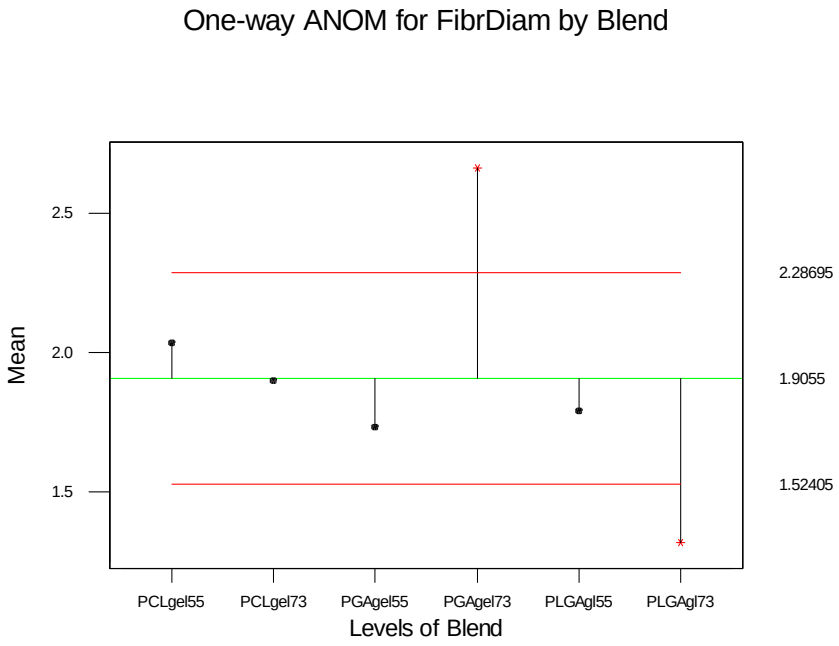
```
Analysis of Variance for Stress
Source     DF        SS        MS        F        P
Blend       6    80.313    13.385    34.62    0.000
Error      35    13.531     0.387
Total      41    93.844
                                 Individual 95% CIs For Mean
                                 Based on Pooled StDev
Level       N      Mean     StDev  ----------+---------+---------+------
gel(con)    6    1.5622    0.2947         (--*---)
PCLGel55    6    2.7703    0.7967                 (--*---)
PCLGel73    6    2.2948    0.5554              (--*---)
PGAGel55    6    0.7168    0.3456   (---*--)
PGAGel73    6    3.7103    0.6732                      (---*--)
PLGAGl55    6    0.6157    0.2645   (--*---)
PLGAGl73    6    4.6003    1.0167                           (---*--)
                                 ----------+---------+---------+------
Pooled StDev =   0.6218                  1.5       3.0       4.5

Tukey's pairwise comparisons

    Family error rate = 0.0500
Individual error rate = 0.00356

Critical value = 4.42

Intervals for (column level mean) - (row level mean)

          gel(con)    PCLGel55    PCLGel73    PGAGel55    PGAGel73    PLGAGl55

PCLGel55     -2.3301
             -0.0862

PCLGel73     -1.8546     -0.6465
              0.3893      1.5975

PGAGel55     -0.2766      0.9315      0.4560
              1.9673      3.1755      2.7000

PGAGel73     -3.2701     -2.0620     -2.5375     -4.1155
             -1.0262      0.1820     -0.2935     -1.8715

PLGAGl55     -0.1755      1.0327      0.5572     -1.0208      1.9727
              2.0685      3.2766      2.8011      1.2231      4.2166

PLGAGl73     -4.1601     -2.9520     -3.4275     -5.0055     -2.0120     -5.1066
             -1.9162     -0.7080     -1.1835     -2.7615      0.2320     -2.8627
```

## One-way ANOVA: Strain versus Blend

```
Analysis of Variance for Strain
Source     DF        SS        MS        F        P
Blend       6   40.7154    6.7859   214.97    0.000
Error      35    1.1048    0.0316
Total      41   41.8203

                                 Individual 95% CIs For Mean
                                 Based on Pooled StDev
Level       N      Mean     StDev  --+---------+---------+---------+----
gel(con)    6    0.0545    0.0136   (-*-)
PCLGel55    6    0.2878    0.0910      (-*)
PCLGel73    6    0.0912    0.0221   (-*-)
PGAGel55    6    0.0567    0.0153   (-*-)
PGAGel73    6    2.6607    0.3479                                  (-*-)
PLGAGl55    6    0.1612    0.0534    (-*-)
PLGAGl73    6    1.8353    0.2965                        (-*-)
                                 --+---------+---------+---------+----
Pooled StDev =   0.1777           0.00      0.80      1.60      2.40

Tukey's pairwise comparisons

    Family error rate = 0.0500
Individual error rate = 0.00356

Critical value = 4.42

Intervals for (column level mean) - (row level mean)

          gel(con)    PCLGel55    PCLGel73    PGAGel55    PGAGel73    PLGAGl55

PCLGel55     -0.5539
              0.0873

PCLGel73     -0.3573     -0.1239
              0.2839      0.5173

PGAGel55     -0.3228     -0.0894     -0.2861
              0.3184      0.5518      0.3551

PGAGel73     -2.9268     -2.6934     -2.8901     -2.9246
             -2.2856     -2.0522     -2.2489     -2.2834

PLGAGl55     -0.4273     -0.1939     -0.3906     -0.4251      2.1789
              0.2139      0.4473      0.2506      0.2161      2.8201

PLGAGl73     -2.1014     -1.8681     -2.0648     -2.0993      0.5047     -1.9948
             -1.4602     -1.2269     -1.4236     -1.4581      1.1459     -1.3536
```

## One-way ANOVA: FibrDiam versus Blend

```
Analysis of Variance for FibrDiam
Source     DF        SS        MS        F        P
Blend       5    29.383     5.877     7.84    0.000
Error     174   130.342     0.749
Total     179   159.724

                                  Individual 95% CIs For Mean
                                  Based on Pooled StDev
Level       N      Mean     StDev  ----+---------+---------+---------+--
PCLgel55   30    2.0340    0.7261              (----*----)
PCLgel73   30    1.8980    0.9277            (-----*----)
PGAgel55   30    1.7330    0.8528          (----*----)
PGAgel73   30    2.6623    1.0652                      (----*-----)
PLGAgl55   30    1.7890    0.7689           (----*----)
PLGAgl73   30    1.3167    0.8084   (----*----)
                                  ----+---------+---------+---------+--
Pooled StDev =   0.8655              1.20      1.80      2.40      3.00

Tukey's pairwise comparisons

    Family error rate = 0.0500
Individual error rate = 0.00441

Critical value = 4.08

Intervals for (column level mean) - (row level mean)

          PCLgel55    PCLgel73    PGAgel55    PGAgel73    PLGAgl55

PCLgel73     -0.5087
              0.7807

PGAgel55     -0.3437     -0.4797
              0.9457      0.8097

PGAgel73     -1.2730     -1.4090     -1.5740
              0.0164     -0.1196     -0.2846

PLGAgl55     -0.3997     -0.5357     -0.7007      0.2286
              0.8897      0.7537      0.5887      1.5180

PLGAgl73      0.0726     -0.0634     -0.2284      0.7010     -0.1724
              1.3620      1.2260      1.0610      1.9904      1.1170
```


One-way ANOM for FibrDiam by Blend
Mean
2.5
2.0
1.5
2.28695
1.9055
1.52405
PCLgel55
PCLgel73
PGAgel55
PGAgel73
PLGAgl55
PLGAgl73
Levels of Blend

# VITA

Alicia P. Smith-Freshwater was born in Fort Bragg, NC on November 24, 1969. The daughter of a career military parent, she has lived in the Republic of the Philippines, Texas, North Carolina and Virginia. Alicia's formative education was provided in the public school systems and universities in North Carolina, Texas and Virginia. She graduated from the NC School of Science and Mathematics in Durham, NC in June 1988 with a high school diploma, after which she became a matriculate at NC Agricultural & Technical State University. After receiving a Bachelor of Science degree in Chemical Engineering in December 1992, Alicia worked for both the private and public sectors as an engineer, trainer, engineering manager and educator for over fifteen years. She was employed with Hoechst-Celanese in Research and Development from 1990 through 1993, Celanese Acetate from 1993 to 2004, the Northampton (NC) and Chesterfield (VA) public school systems from 2004 through 2009 and at Virginia Commonwealth University from 2006 through 2008. Alicia began her graduate studies at Virginia Commonwealth University (VCU) in Biomedical Engineering in the Fall 2006 session, and was inducted into the Alpha Eta Mu Beta Biomedical Engineering Honor Society in Spring 2007. Throughout her matriculation at VCU, she has acquired invaluable career experience and training as a Graduate Teaching Assistant, Laboratory Manager, Southern Regional Education Board Doctoral Fellow, VCU BBSI Summer Research Institute mentor for undergraduate interns and as a presenter at various scientific symposiums.